



# Laboratory performance in the detection of Weaker Expression of ABO Blood Group Antigens - a comparison of Educational Exercises from 2004 and 2008"

Arthur Joyce Mater Pathology Brisbane

Allan Sandercock RCPA Transfusion QAP

Neville Hermann RCPA Transfusion QAP



## Blood Grouping – Lab Performance

- QAP Blood Grouping
  - ABO – 90-100% correct
    - AG/AB 2009-4 Patient Group A Neg
    - -1/366 Returns error in ABO Group
    - (Neg result with B cells in Reverse Group)
  - Errors Transcription – Reverse Group
  - Anti-A, Anti-B errors minimal
- Weaker Expression of A & B antigens
  - Expect no difficulties



## Educational Exercises – Blood Grouping

- 2004 x 4 samples
  - 3 Group A and 1 Group B
- 2008 repeat exercise –
  - 3 Group A and 1 Group B



# Educational Exercises – Blood Grouping

Samples supplied by CSL Immunohaematology all “manufactured” using the KODE process –

Group O cells with predetermined amounts of A and B antigen inserted into the membrane

Created 3 weak A cells and a weak B cell with desired levels of antigen expression

$A_2, A_3, A_x, B_{\text{weak}}$



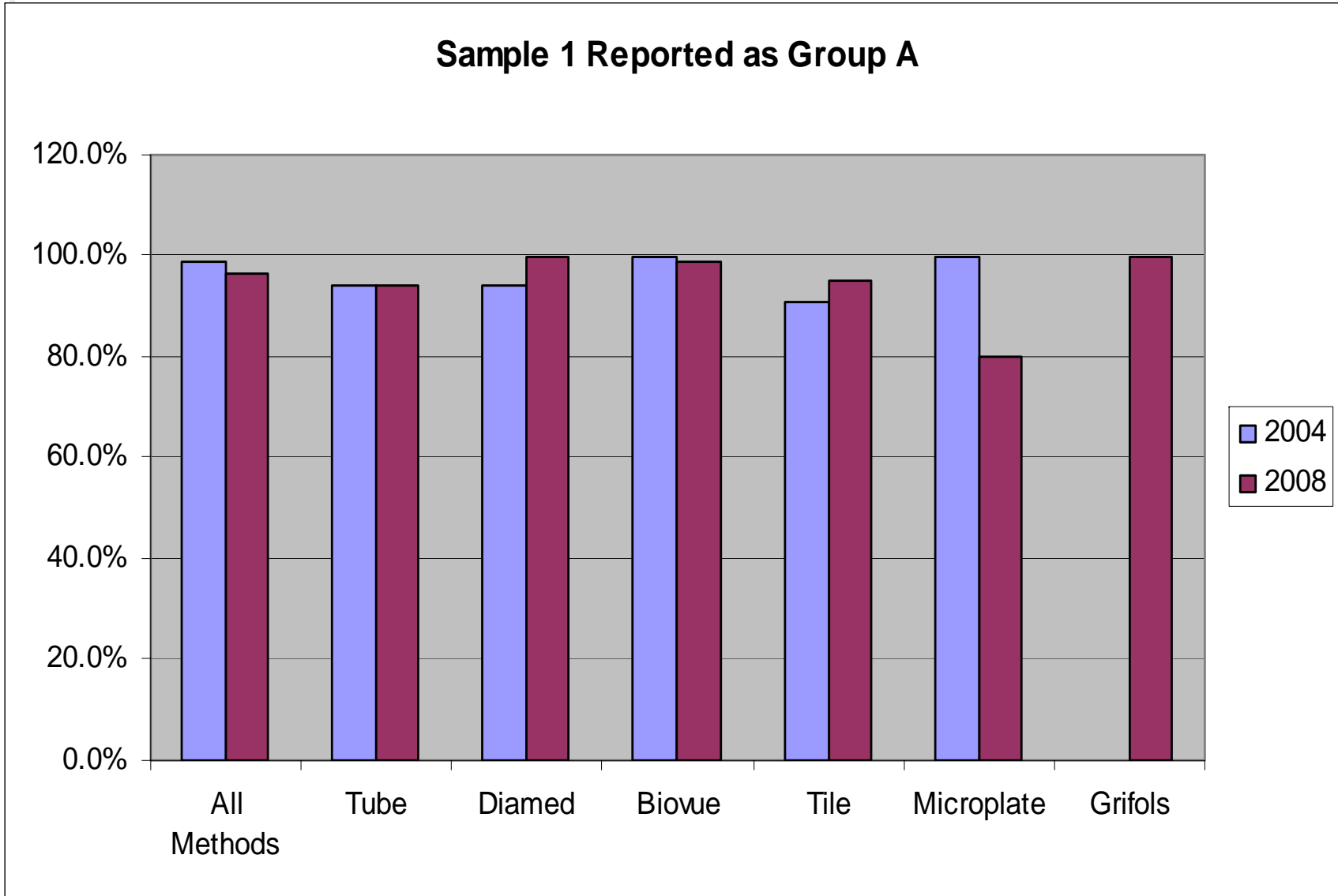
# Average Scores

## A2004-2 Educational Exercise

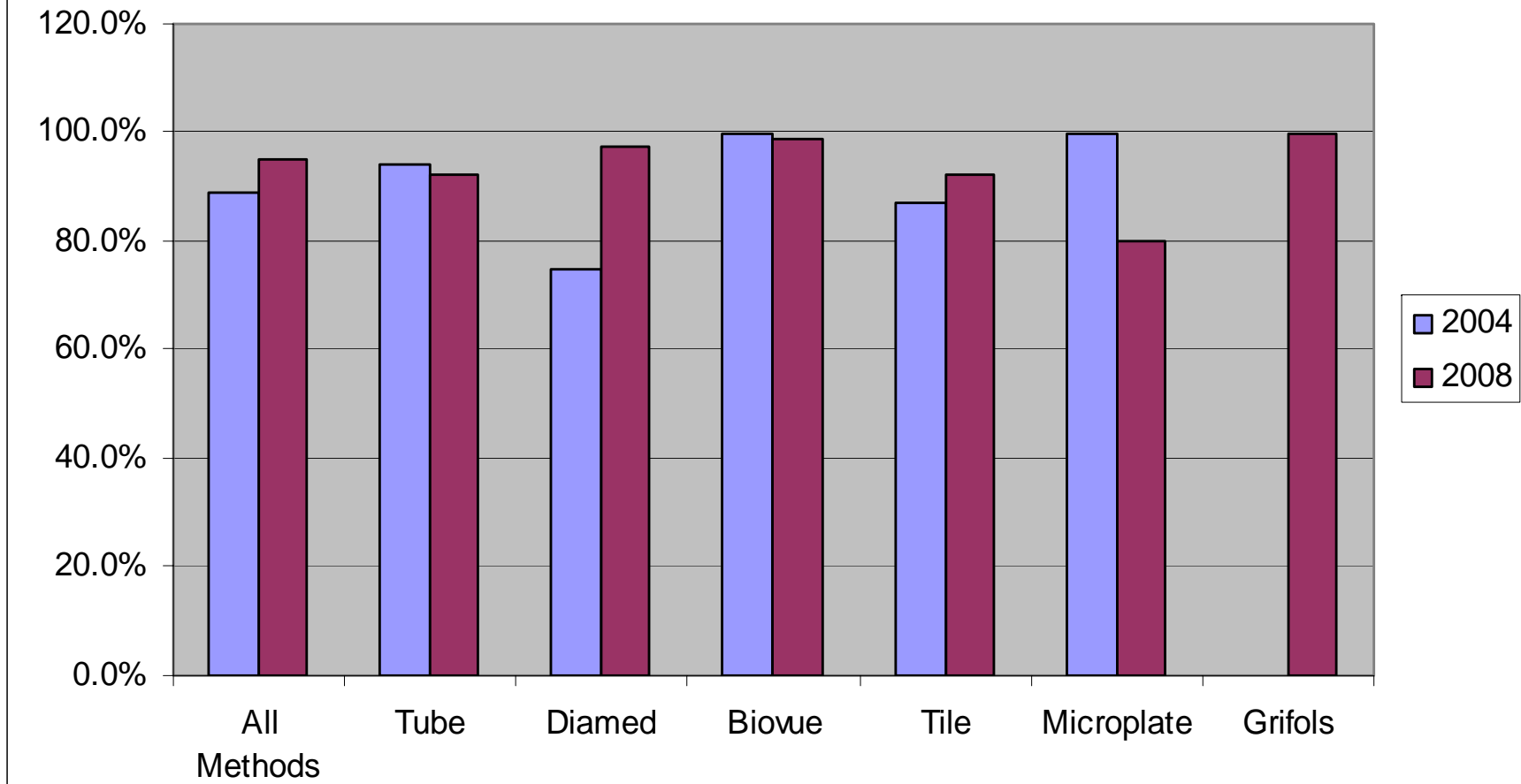
Sample Averages						
	Antisera			Cells		Group Rh(D)
	A	B	D	A1	B	
Sample 1	9	0	0	0	11	A Neg
Sample 2	7	0	12	0	11	A Pos
Sample 3	1	0	12	0	11	A Pos
Sample 4	0	8	12	11	0	B Pos

## A 2008-6 Educational Exercise

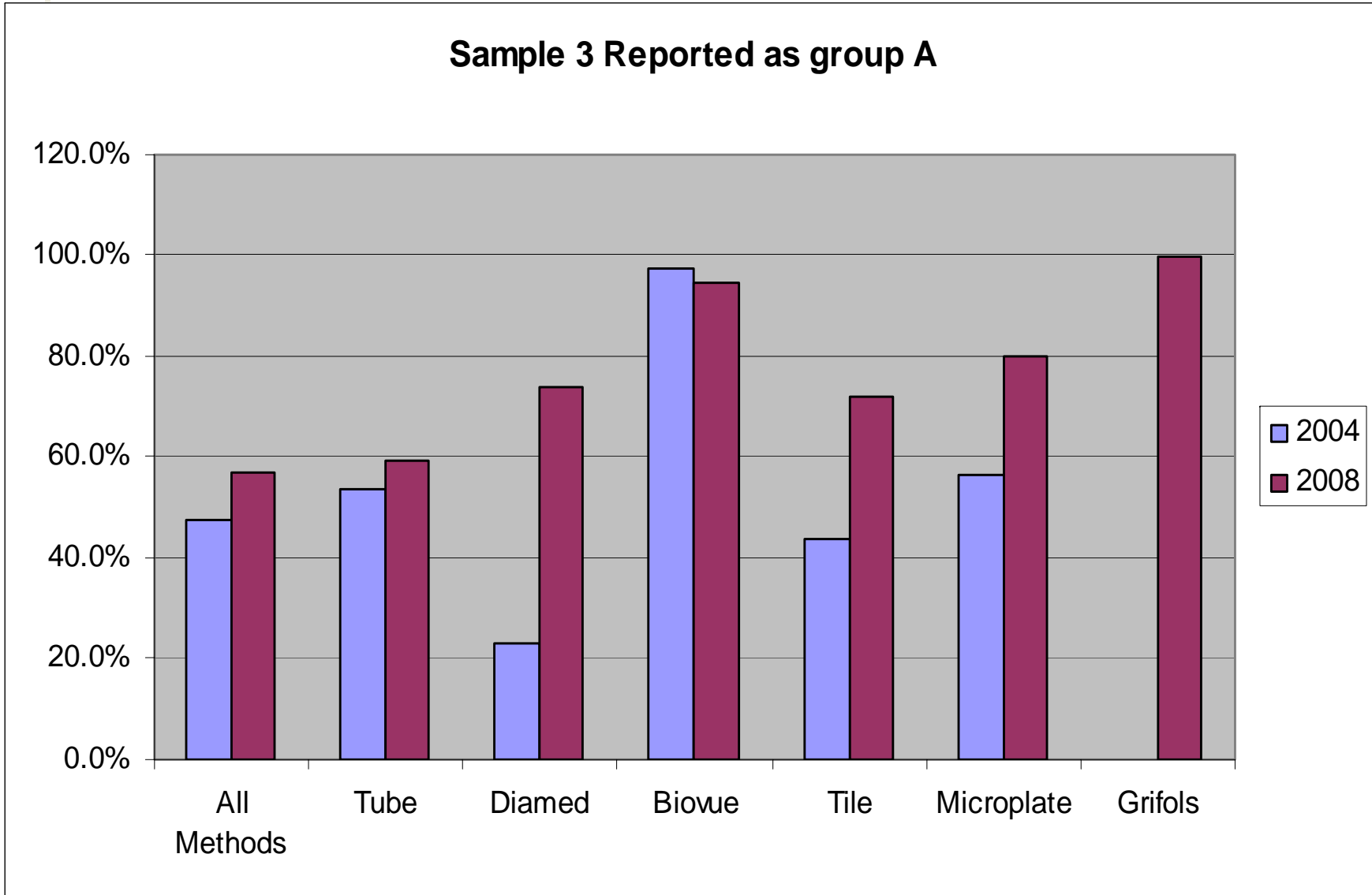
Sample Averages						
	Antisera			Cells		Group Rh(D)
	A	B	D	A1	B	
Sample 1	9	0	12	0	11	A Pos
Sample 2	8	0	12	0	11	A Pos
Sample 3	2	0	12	0	12	A Pos
Sample 4	0	9	0	11	0	B Neg



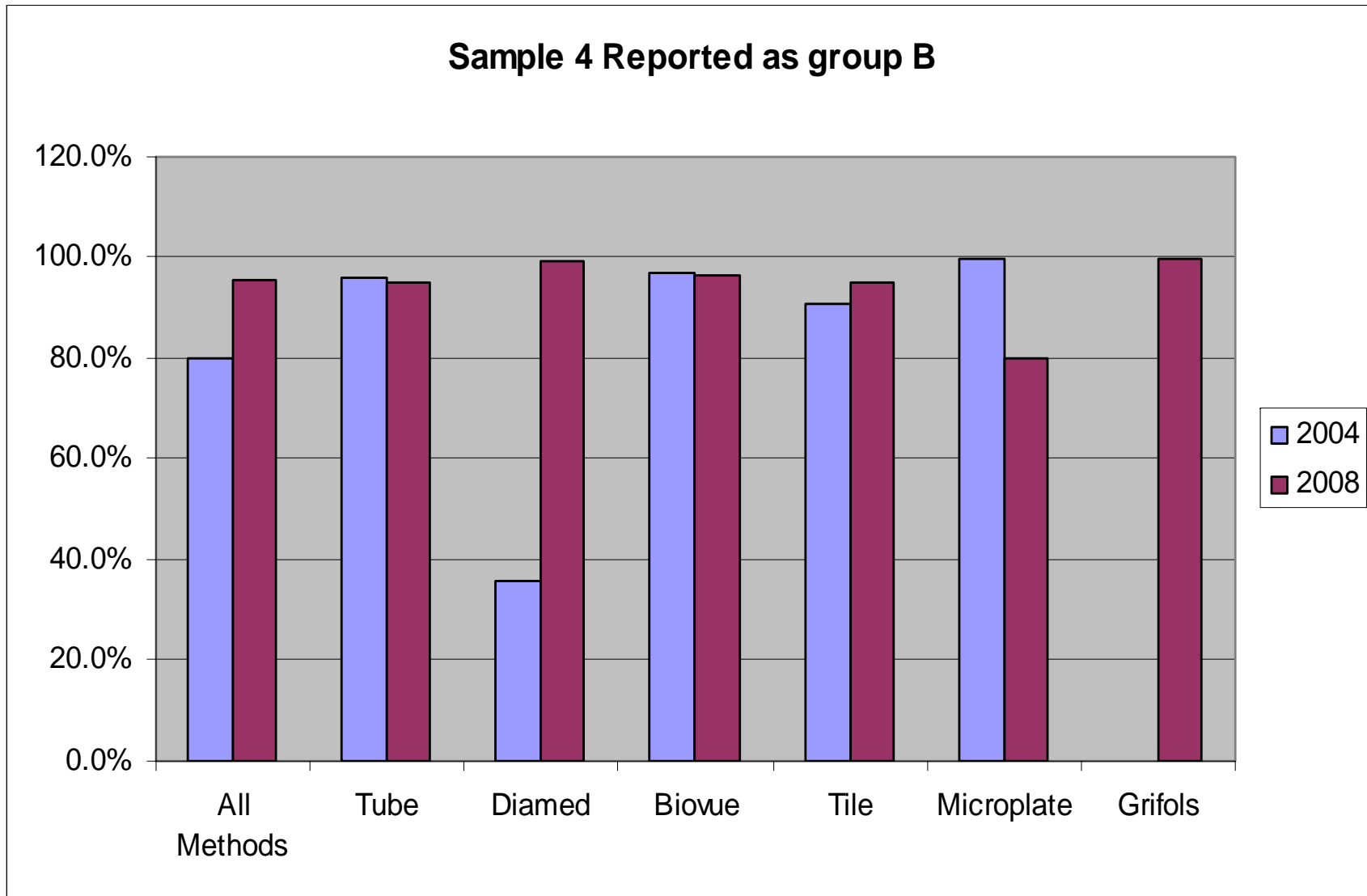
### Sample 2 Reported as group A



### Sample 3 Reported as group A



### Sample 4 Reported as group B





# Interpretation

## A2004-2 Educational Exercise

Sample 1		A	511	518	99%	
Sample 2		A	461	518	89%	
Sample 3		A	249	522	<b>48%</b>	
Sample 4		B	415	518	80%	

## A 2008-6 Educational Exercise

Sample 1		A	501	519	97%	
Sample 2		A	499	524	95%	
Sample 3		A	301	527	<b>57%</b>	
Sample 4		B	501	524	96%	



## Sample 3 -Gel Technology Diamed

2004

- Anti-A Score 0 = 82/83 (99%)
- Group A Interpretation = 23%

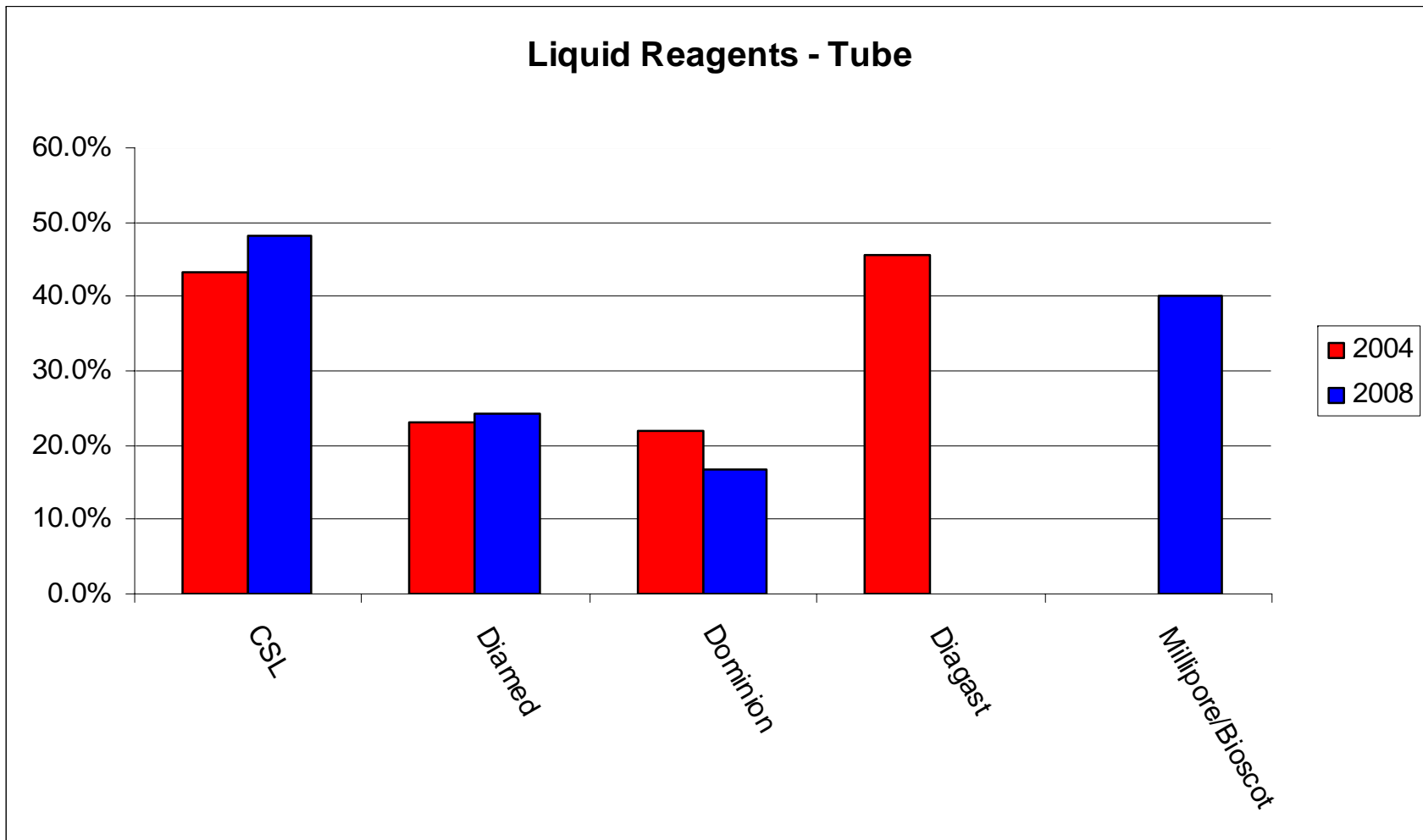
2008

- Anti-A Score 0 = 102/105 (97%)
- Group A Interpretation = 74%

## Tube –Liquid Antisera Various

- 2004
  - Anti-A Score 0 = 42/67 (63%)
  - Group A Interpretation = 54%
  
- 2008
  - Anti-A Score 0 = 45/77 (58%)
  - Group A Interpretation = 59%

# Sample 3 – Tube Pos Manufacturer

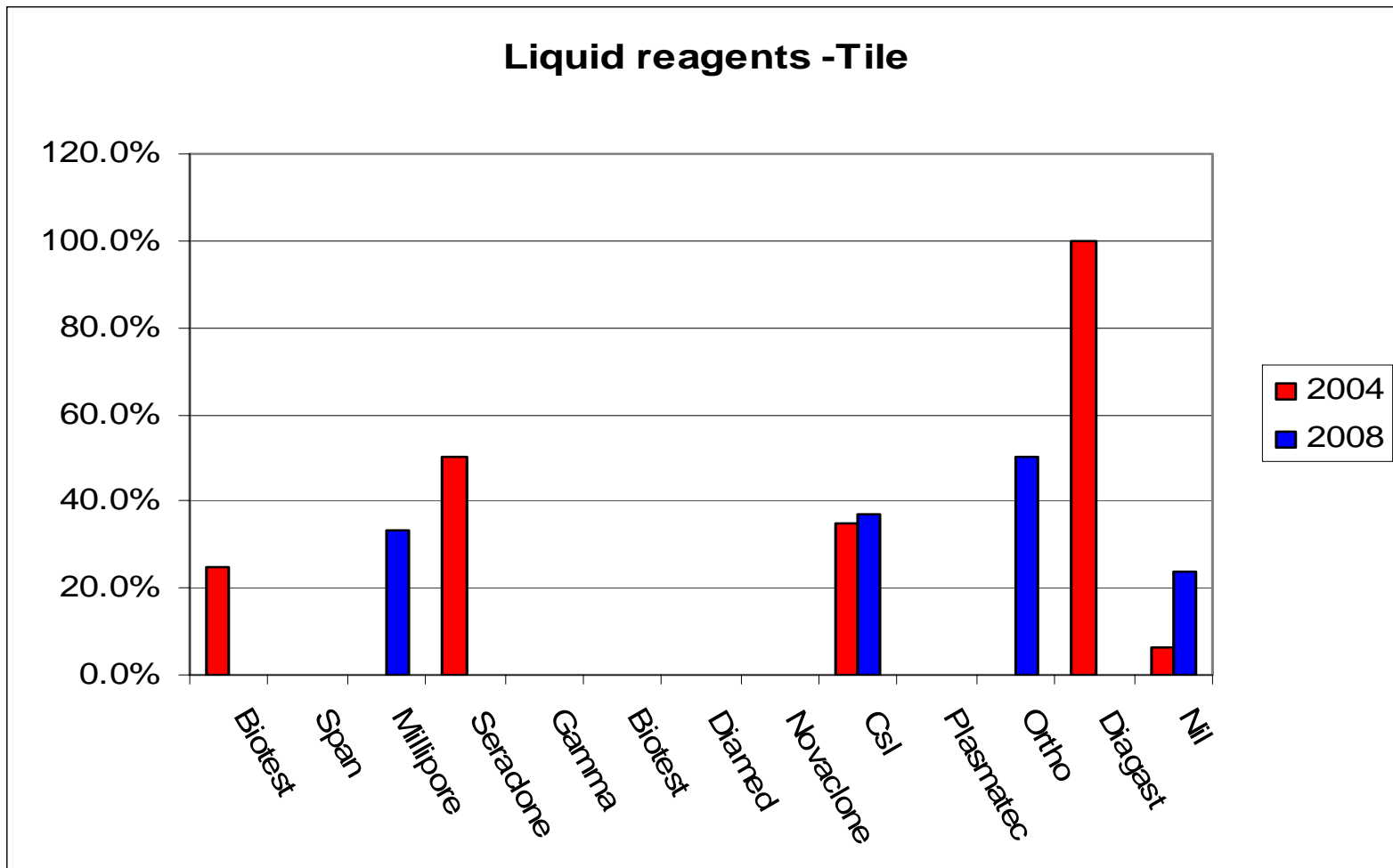




## Tile – Liquid Antisera (Various)

- 2004
  - Anti-A Score 0 = 29/38 (76%)
  - Group A Interpretation = 43%
  
- 2008
  - Anti-A Score 0 = 35/49 (71%)
  - Group A Interpretation = 72%

# Sample 3 Tile pos by M'factorer





# CAT – BioVue OCD

- 2004
  - Anti-A Score 0 = 0/6 (0%)
  - Group A Interpretation = 100%
  
- 2008
  - Anti-A Score 0 = 0/31 (0%)
  - Group A Interpretation = 94%



# Reagent Validation

- ANZSBT guidelines –
  - Quality Control of Reagents -
    - Anti-A testing with A cells and
    - Anti-B tested with B cells
- NPAAC Requirements –
  - Quality Control of Reagents –
    - Nil



# MINIMUM TEST CELLS FOR SPECIFICITY

## REAGENT RED BLOOD CELLS

- Anti-A             $A_1$  (1) and  $A_2B$  \* (3)
- Anti-B            B (3) and  $A_2 B$  (1)
- Anti-A,B         $A_1$  (2)  $A_2$  \*\* (2), and B (4) and at least three different  $A_x$  \*\*\*

\* AB cells which do not react with anti- $A_1$  and do react with anti-H.

\*\* A cells which do not react with anti- $A_1$ , and do react with anti-H

\*\*\*  $A_x$  cells are recommended; labelling should indicate detection of group A variants.

Include examples of "strong  $A_x$  cells" and "moderate  $A_x$  cells". "Weak  $A_x$  cells" are optional.

FDA March 1992



# MINIMUM TEST CELLS FOR POTENCY

## REAGENT RED BLOOD CELLS

- Anti-A             $A_1$  (1) and (3) different  $A_2B$  \*
- Anti-B            B and  $A_1 B$
- Anti-A,B         $A_1 A_2$  \*\* and B

\* AB cells which do not react with anti- $A_1$  and do react with anti-H.

\*\* A cells which do not react with anti-  $A_1$ , and do react with anti-H

FDA March 1992

# MINIMUM TEST CELLS FOR AVIDITY

## REAGENT RED BLOOD CELLS

- Anti-A  $A_1$  (1) and  $A_2B^*$  (3)
- Anti-B B (3) and  $A_2B$  (1)
- Anti-A,B  $A_1$  (2)  $A_2^{**}$  (2), and B (4) and at least three different  $A_x^{***}$

\* AB cells which do not react with anti- $A_1$  and do react with anti-H.

\*\* A cells which do not react with anti- $A_1$ , and do react with anti-H

\*\*\*  $A_x$  cells are recommended only if the reagent is recommended for detection of weak subgroups of A by slide technique

FDA March 1992



# Reactivity & Specificity

- No immune haemolysis, rouleaux or prozone phenomenon
- Clear cut reactions with RBC bearing the weakened expression of the corresponding antigen (s), no false reactions.
- CoE 2007



# Potency

- Undiluted reagent should give a 3 to 4 plus reaction in saline tube test using a 3% RBC suspension at room temperature.

- CoE 2007



# Other Considerations

- Evaluation of quality performed on reagents before purchase
- Suppliers provide full validation data
- Reagents validated by purchaser compare to that provided by manufacturer
- Minimum potency standards for Anti-A , Anti-B and Anti-D should be used in the assessment of blood grouping reagents

# Subgroups of Blood Group A

A Subtype	Frequency of Group A Phenotypes	Reactions With Monoclonal Anti-A Typing sera	Reactions With Monoclonal Anti A,B typing sera
A <sub>1</sub>	78 %	Strong Positive	Strong Positive
A <sub>2</sub>	8 - 10 %	Strong Positive	Strong Positive
A <sub>3</sub>	1:1000 - 1:150,000	Mixed Field Reactions	Mixed Field Reactions
A <sub>x</sub>	1:40,000- 1:77,000	Negative	Weak Agglutination
A <sub>end</sub>	1:75,000	Very Weak Positive	Very Weak Positive
A <sub>el</sub>	Rare	Negative, though some A <sub>el</sub> B cells are agglutinated by monoclonal anti-A reagents	Negative
A <sub>m</sub>	Rare	Negative / Weak Positive	Negative / Weak Positive
A <sub>y</sub>	Rare	Negative	Negative



# Summary

- Should Anti-A Reagents used today detect weakly reacting sub groups? (Anti-A,B not used in many blood grouping panels)
- If Yes – what specificity?
- Supply of A<sub>x</sub> cells for QC testing limited
- Are manufactured cells eg KODE cells suitable for laboratory QC?
- Should the QAP include Cells with weaker expression of A&B antigens into the QAP?

# Educational Exercise 2009A

