

# Transfusion medicine & KODE™ technology: R&D tools, QC systems, teaching, diagnostics and therapeutics



NATURE July 23, 1955 VOL 176

Transformation of the Lewis Groups of Human Red Cells

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ADSORPTION OF BLOOD-GROUP SUBSTANCES FROM SERUM ON TO RED CELLS

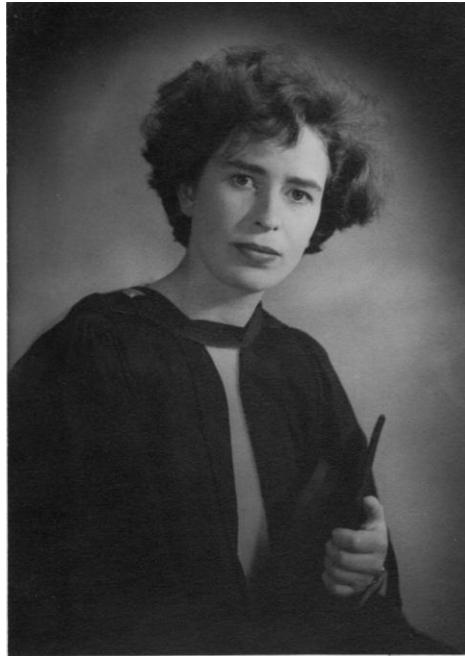
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Medical Research Council  
Blood Group Research Unit  
Lister Institute of Preventive Medicine, London

National Institute for Medical Research  
Mill Hill, London

Brit. Med. Bull. 1959



Joan Sylvia Thompson, 1950



Lafayette Ltd  
Peter Henry Andrews Sneath Circa 1950

19xx - 2005

1923 - 2011

"What marvelous things have resulted from the early work of the three teams at the Lister Institute, led by Rob Race and Ruth Sanger, Walter Morgan and Winifred Watkins, and Arthur Mourant and Elizabeth Iken!"

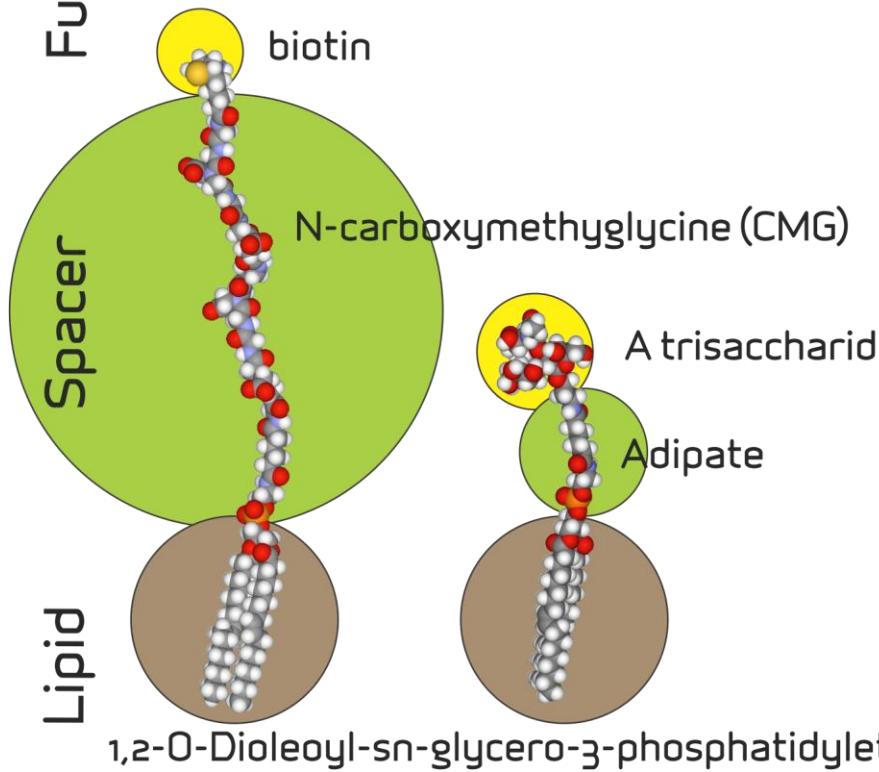
..... and certainly you may refer to me as grandfather of KODE technology, but if so my wife Joan is a grandmother!"

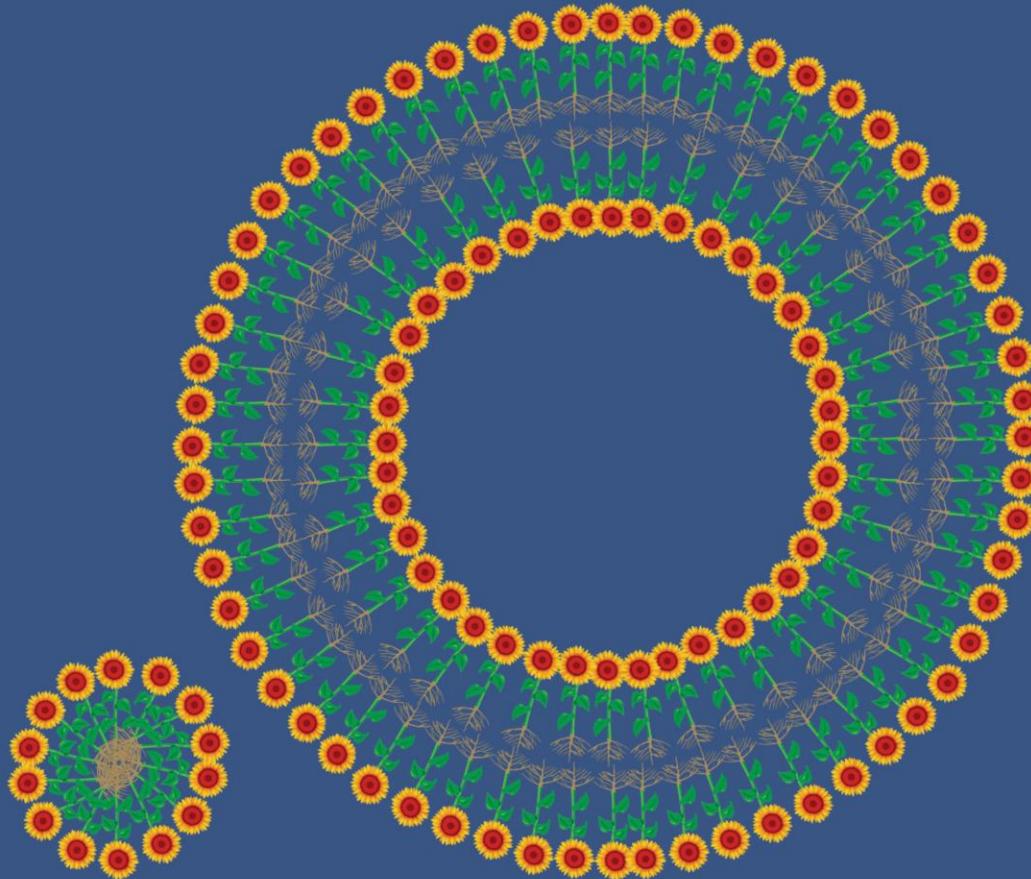
- Peter Sneath 2009



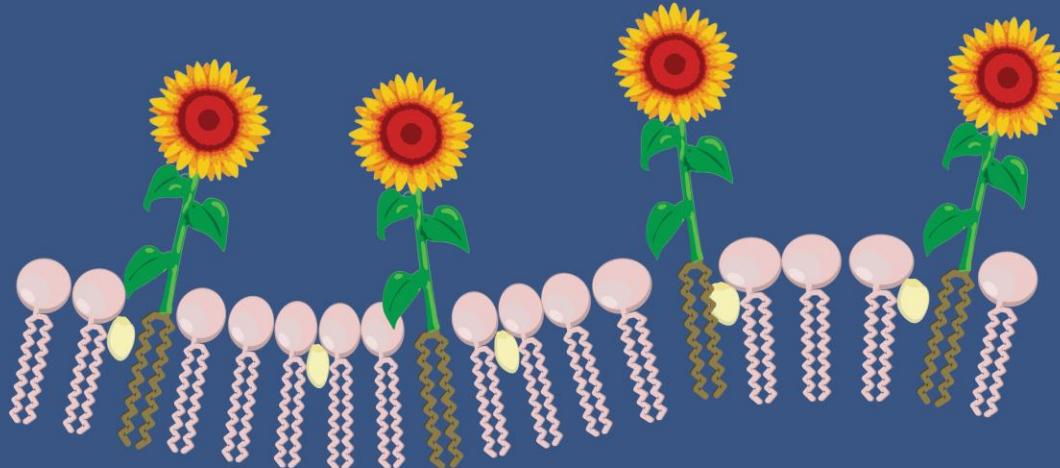
## FSL constructs

Function  
Spacer  
Lipid

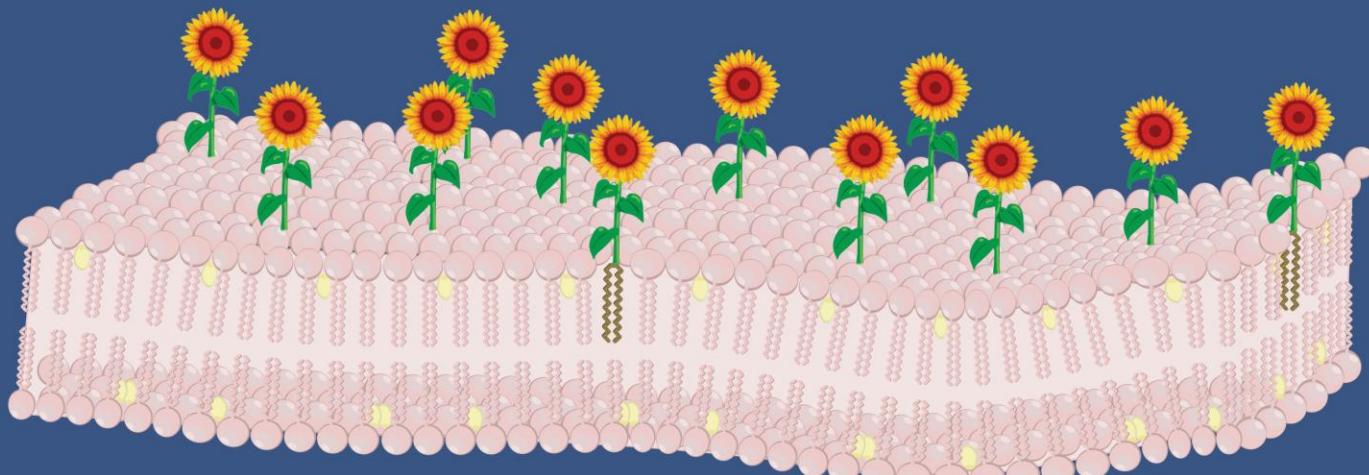


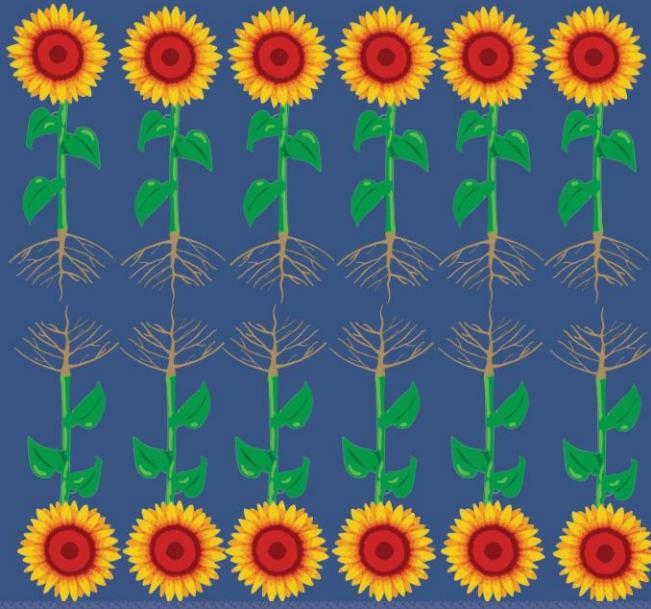


solution phase FSL micelles



lipid membrane insertion





hydrophilic coating



hydrophobic coating



carbohydrates

polysaccharide

peptides

radio-isotopes

maleimide

charge

sialic acid

antibodies

hyaluronic acid

fluorophores

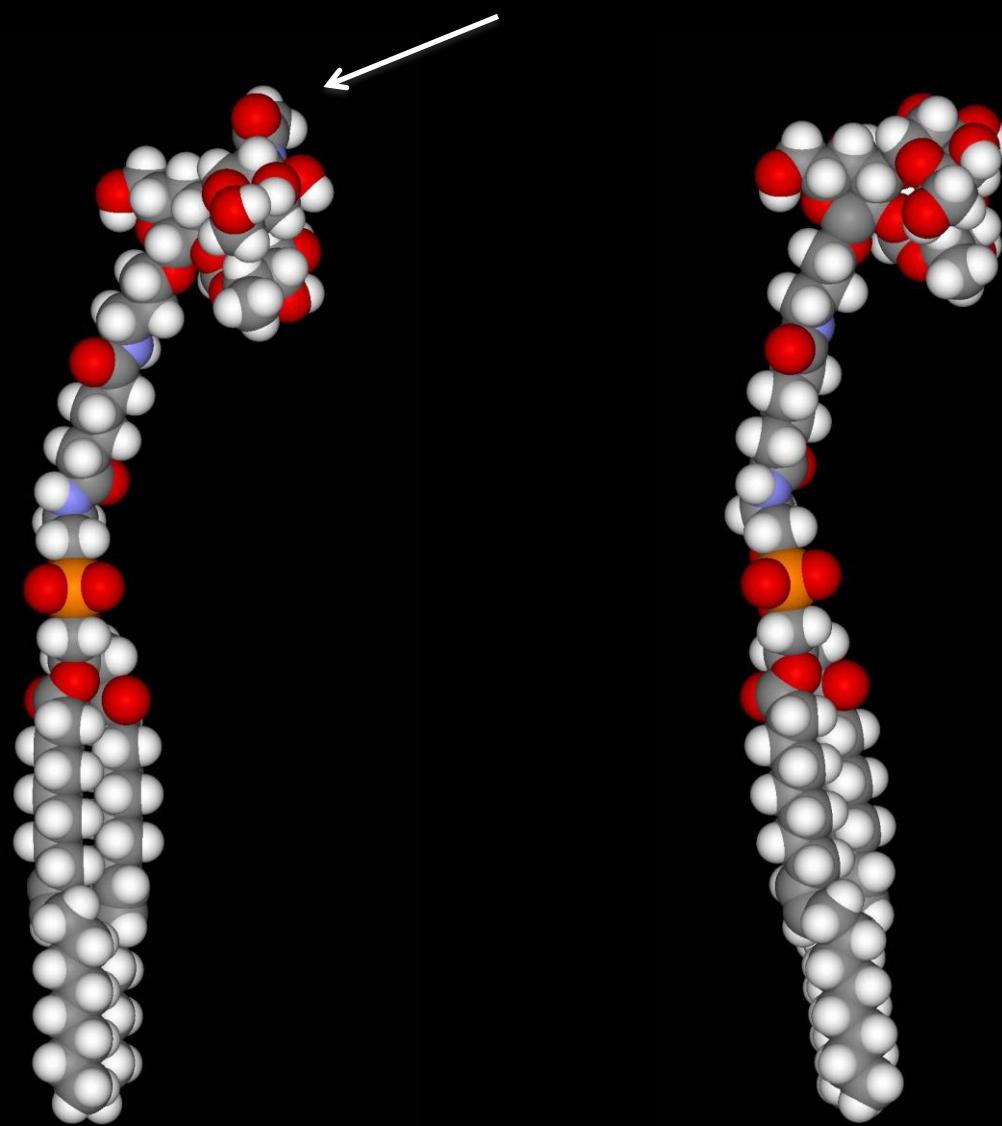
biotin

click

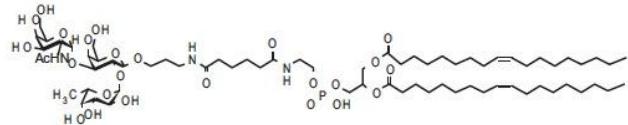
PEG

etc

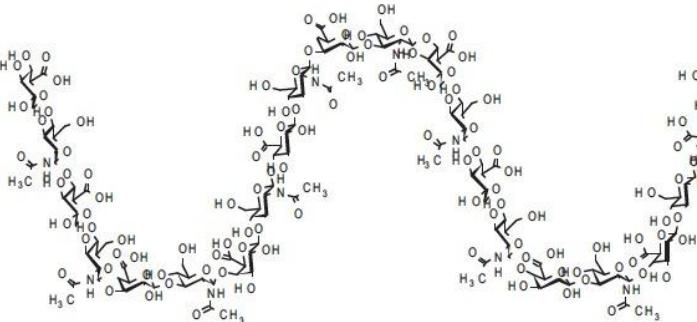
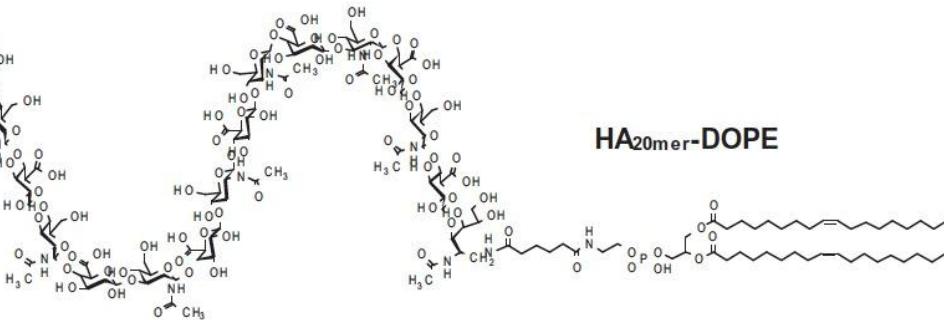
# Function-Spacer-lipid constructs of blood group A & B (trisaccharides)



**A<sub>tri</sub>-Ad-DOPE**



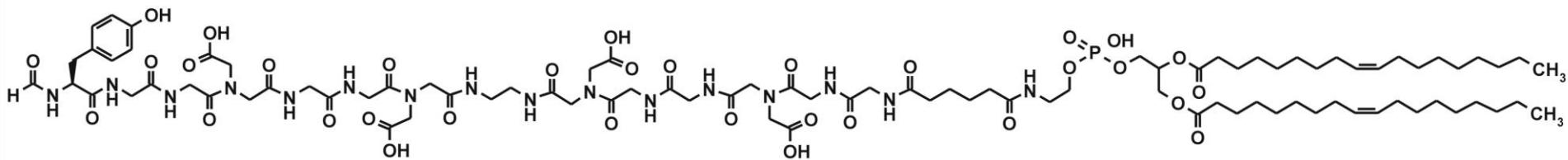
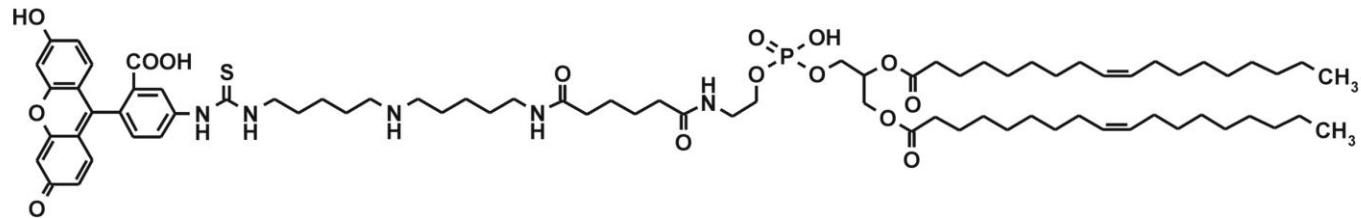
**HA<sub>20mer</sub>-DOPE**



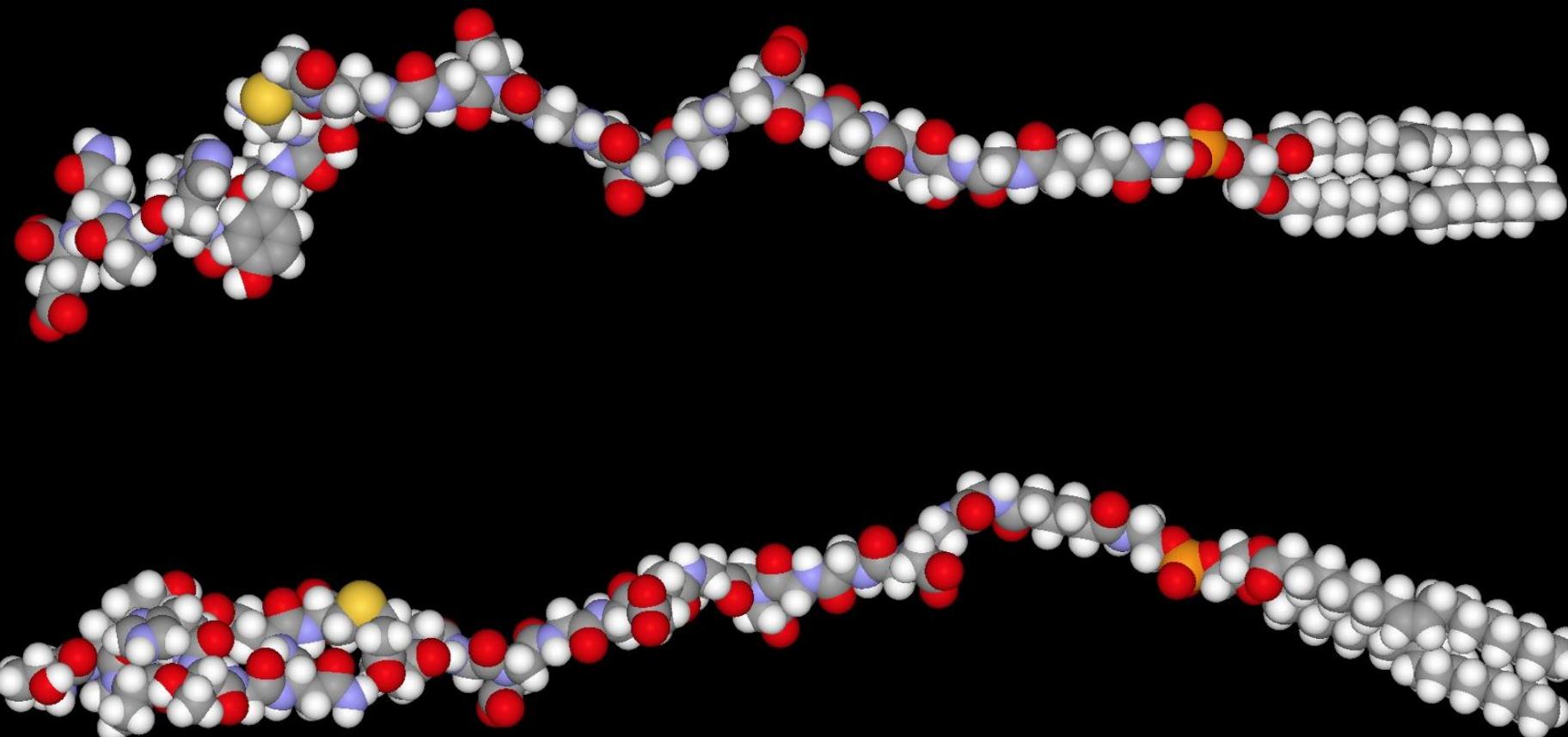
**Chart 3.** Scale comparison of FSL-A with FSL-HA. FSL-HA is 40 oligosaccharide (~8 kDa), whereas FSL-A<sub>tri</sub> is a trisaccharide.

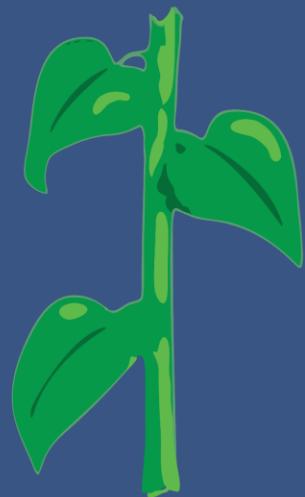
## FSL-FLRO4(fluorescein)

# FSL-tyrosine



# FSL peptides





1.9nm (Ad)

11.5nm (CMG4)

**biologically inert**

unreactive with undiluted serum

flexible

optimizes presentation

polarity

rigid

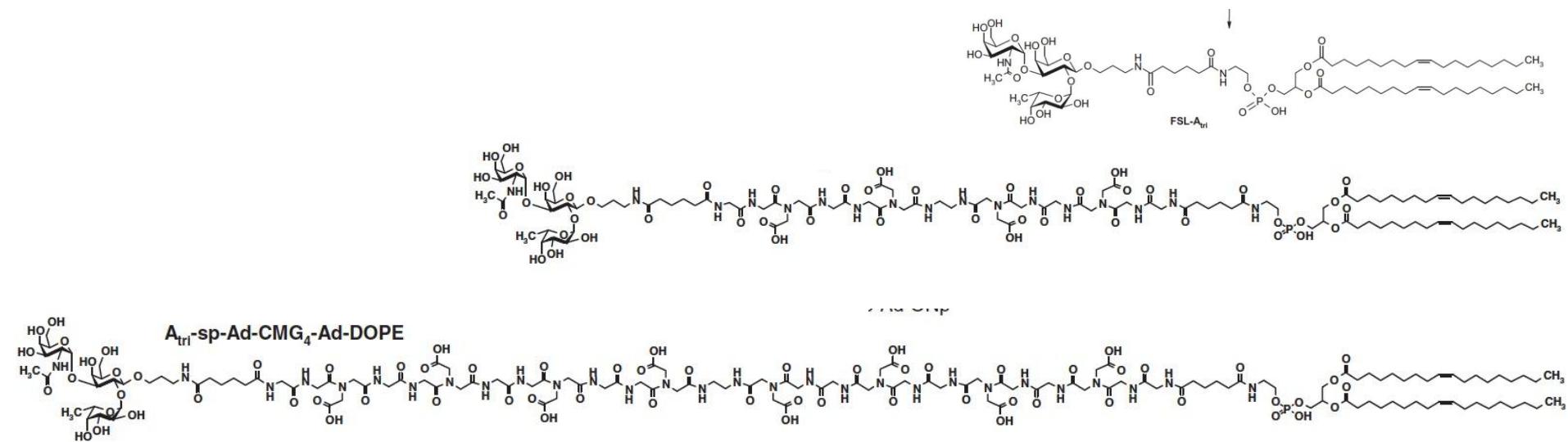
charge

linear

spacing

branched

7.2nm (CMG2)



**Table 4**

Serologic reactions of blood group A kodescetes created with different FSL spacers

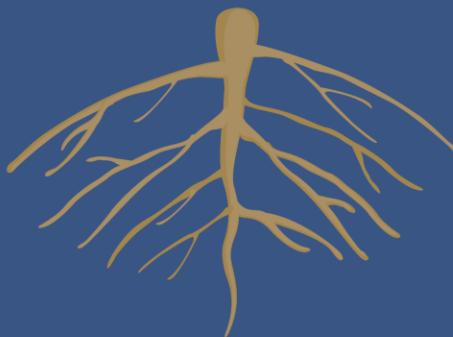
FSL-A <sub>tri</sub> <sup>a</sup>		FSL-A kodescete serologic reactions <sup>b</sup> against dilutions of monoclonal anti-A										
Spacers <sup>c</sup>	μM	1	2	4	8	16	32	64	128	256	512	1024
Ad	50	++++	++++	++++	++++	++++	++++	+++	++	++	±	—
	10	+++	+++	+++	++	++	++	++	±	—	—	—
	5	++	++	+	±	—	—	—	—	—	—	—
CMG <sub>2</sub>	50	++++	++++	++++	++++	++++	++++	+++	+++	++	+	±
	10	++++	++++	++++	+++	+++	+++	+++	++	+	±	—
	5	+++	+++	+++	+++	++	++	++	+	±	—	—
CMG <sub>4</sub>	50	++++	++++	++++	++++	++++	++++	+++	+++	++	+	±
	10	++++	++++	++++	+++	+++	+++	+++	++	++	±	—
	5	+++	+++	+++	+++	++	++	++	+	±	—	—

<sup>a</sup> All FSL constructs have A trisaccharide and DOPE as the lipid tail.

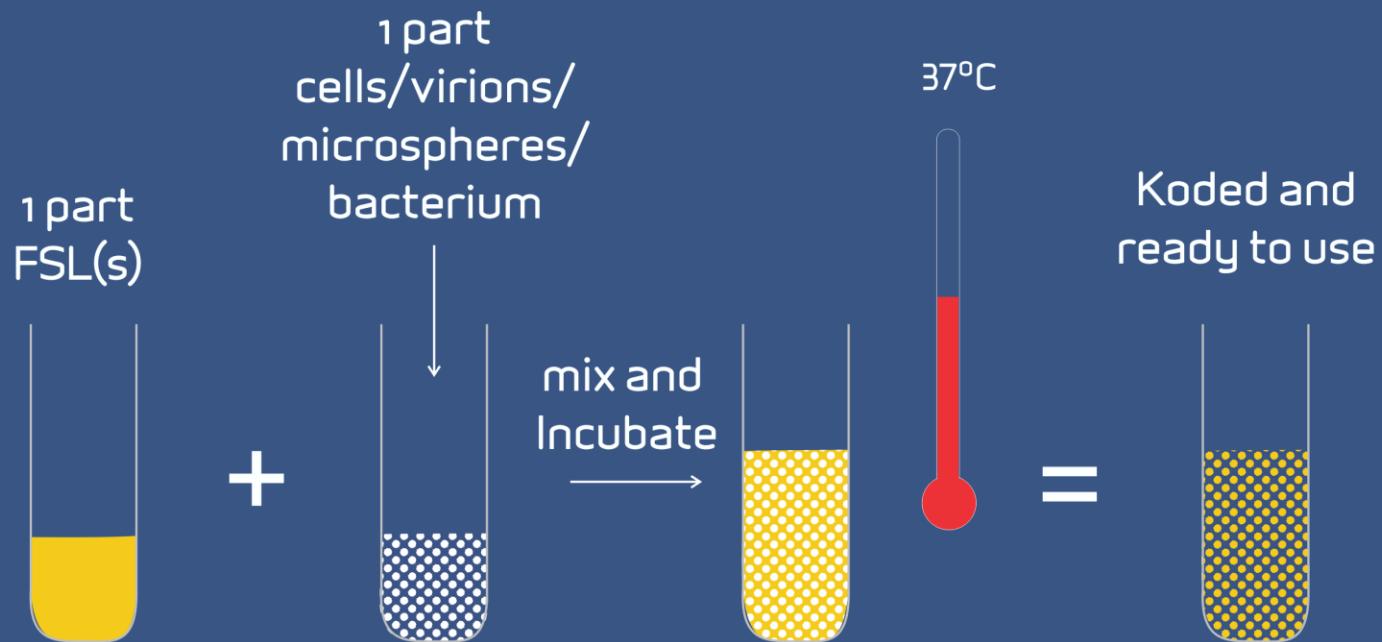
<sup>b</sup> Serologic reactions observed against dilutions of monoclonal anti-A in gel reaction cards and scored as ++++ (maximal agglutination), +++ (strong), ++ (medium), + (weak), ± (equivocal) and — (unreactive).

<sup>c</sup> Spacers are as described in Chart S2.

diacyl      DOPE  
diakyl  
  
sterol      cholesterol  
ceramide

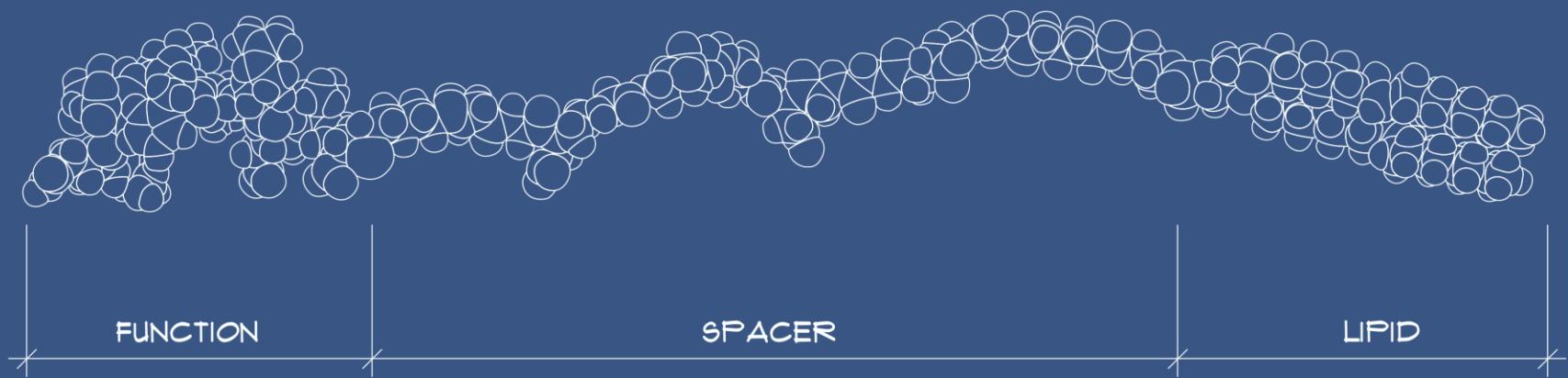


## A novel bioactive surface within 2 hours



# KODE™ technology

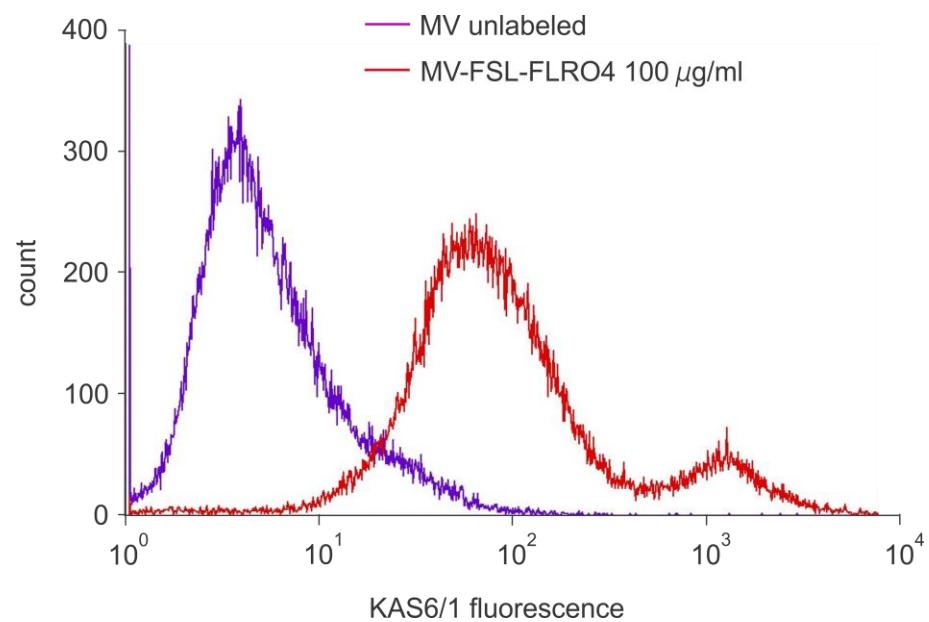
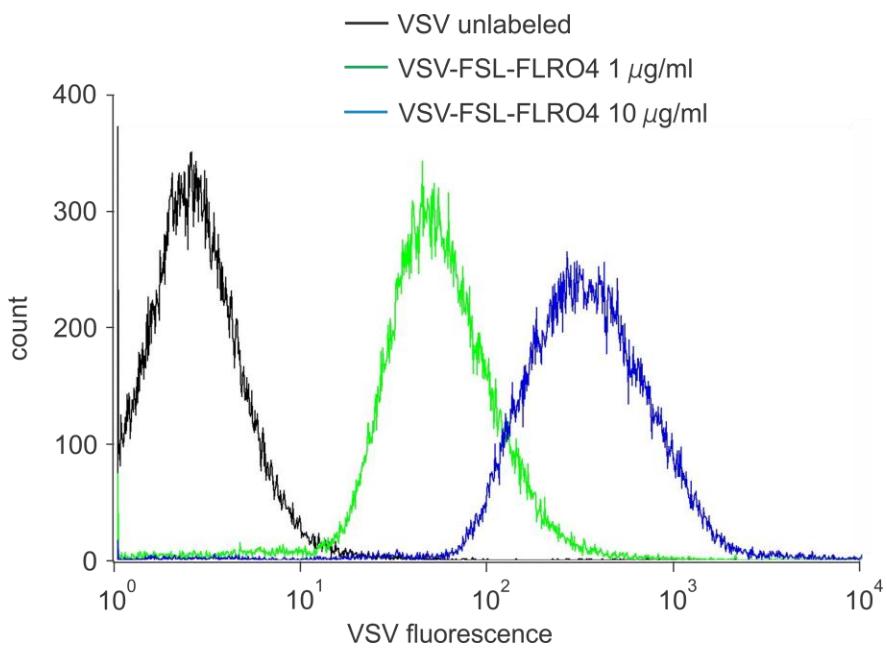
## R&D tools



Established applications

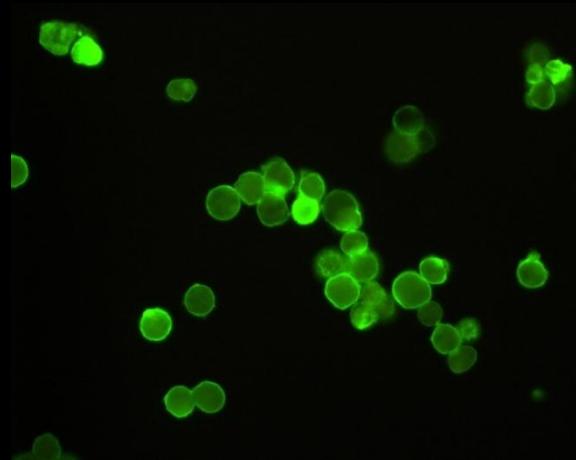
**cells      surfaces**  
organisms      bacteria  
**viruses**      vesicles

# FLRO4 kodevirions

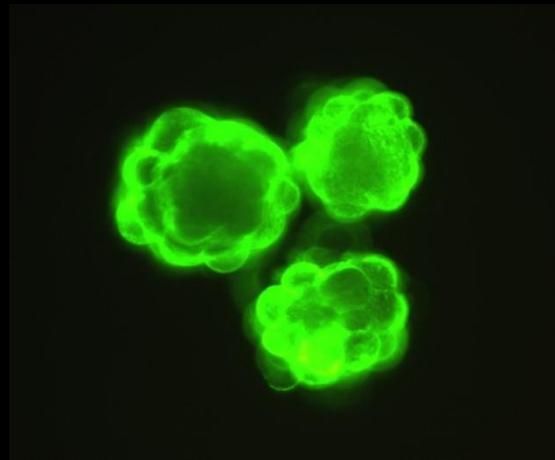


# Surface antigen labeling

(FSL-A + anti-A then secondary anti-Ig fluorescein detection)



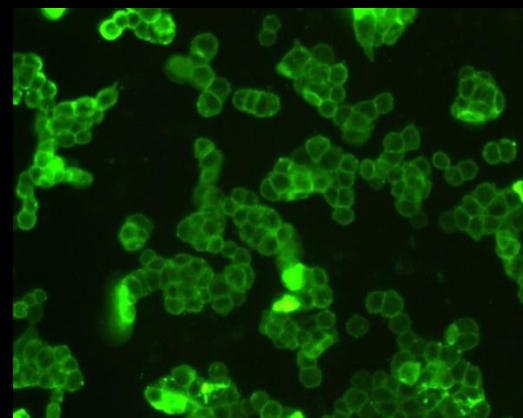
Fixed endometrial cells



Hatched embryos

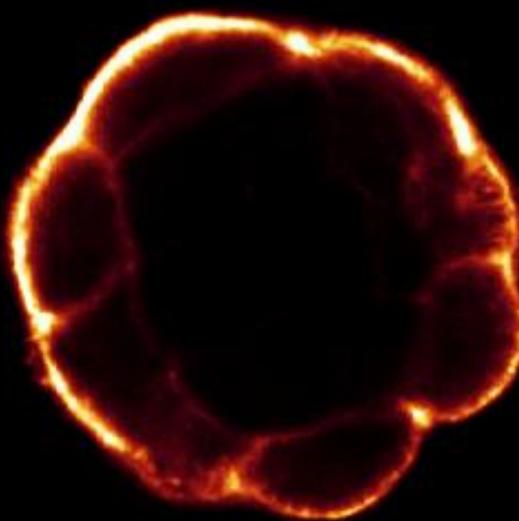


RL95 endometrial cell line



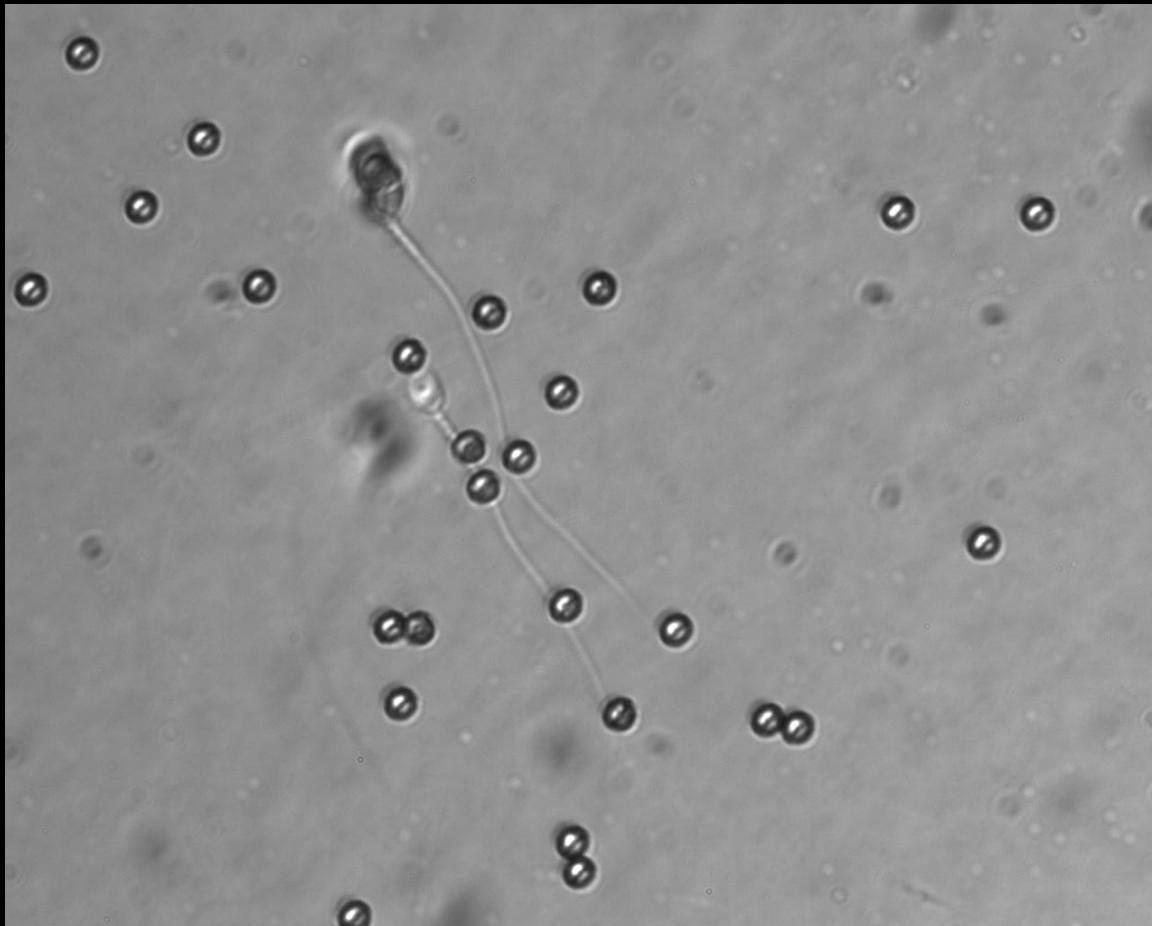
# Confocal biotin embryo kodecyte

(secondary label – avidin-Alexafluor®)



# kodecyte adhesion

(biotin sperm kodecytes and streptavidin-coupled DynaBeads®)



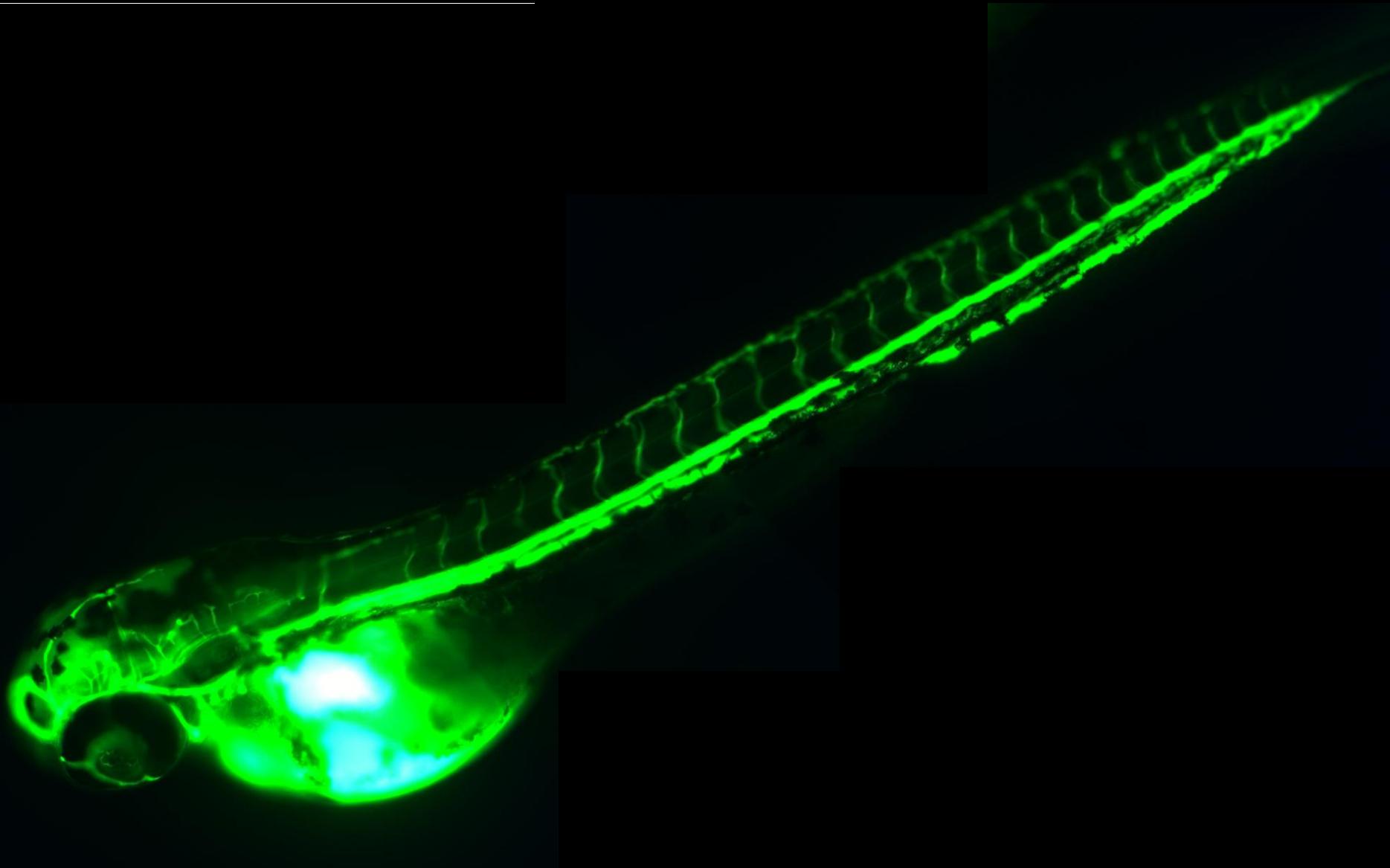
Rate of attachment <1 sec

Cells immobilised but  
viability unaffected

# Injected

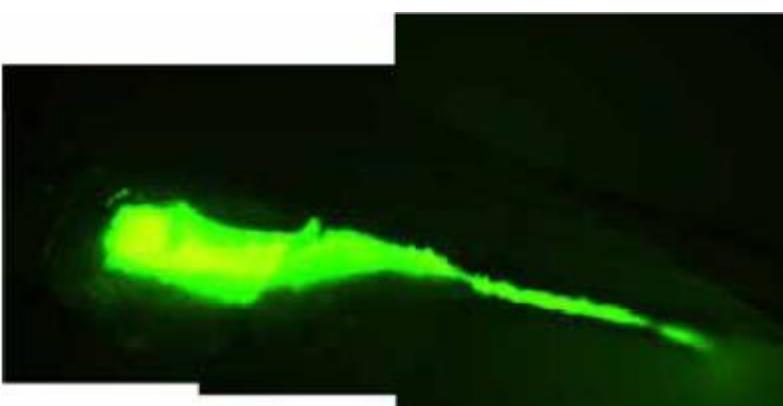
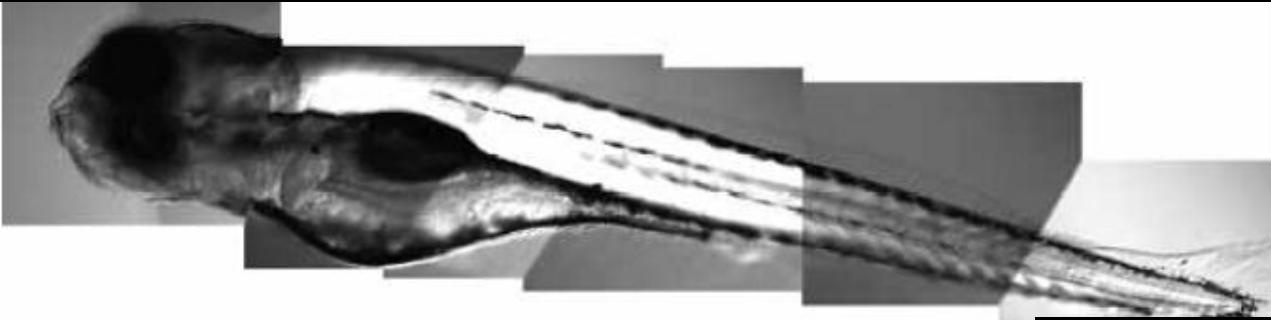
FSL-FLRO4 infusion in Zebra fish

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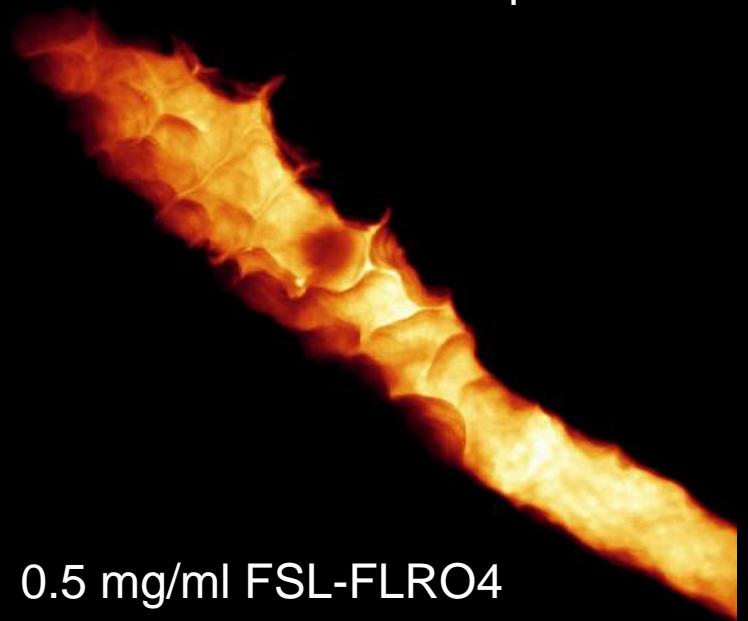


# FSL-FLRO4 ingestion

Zebra fish embryos swum in FSL-FLRO4



0.1 mg/ml FSL-FLRO4  
-14 hrs post wash

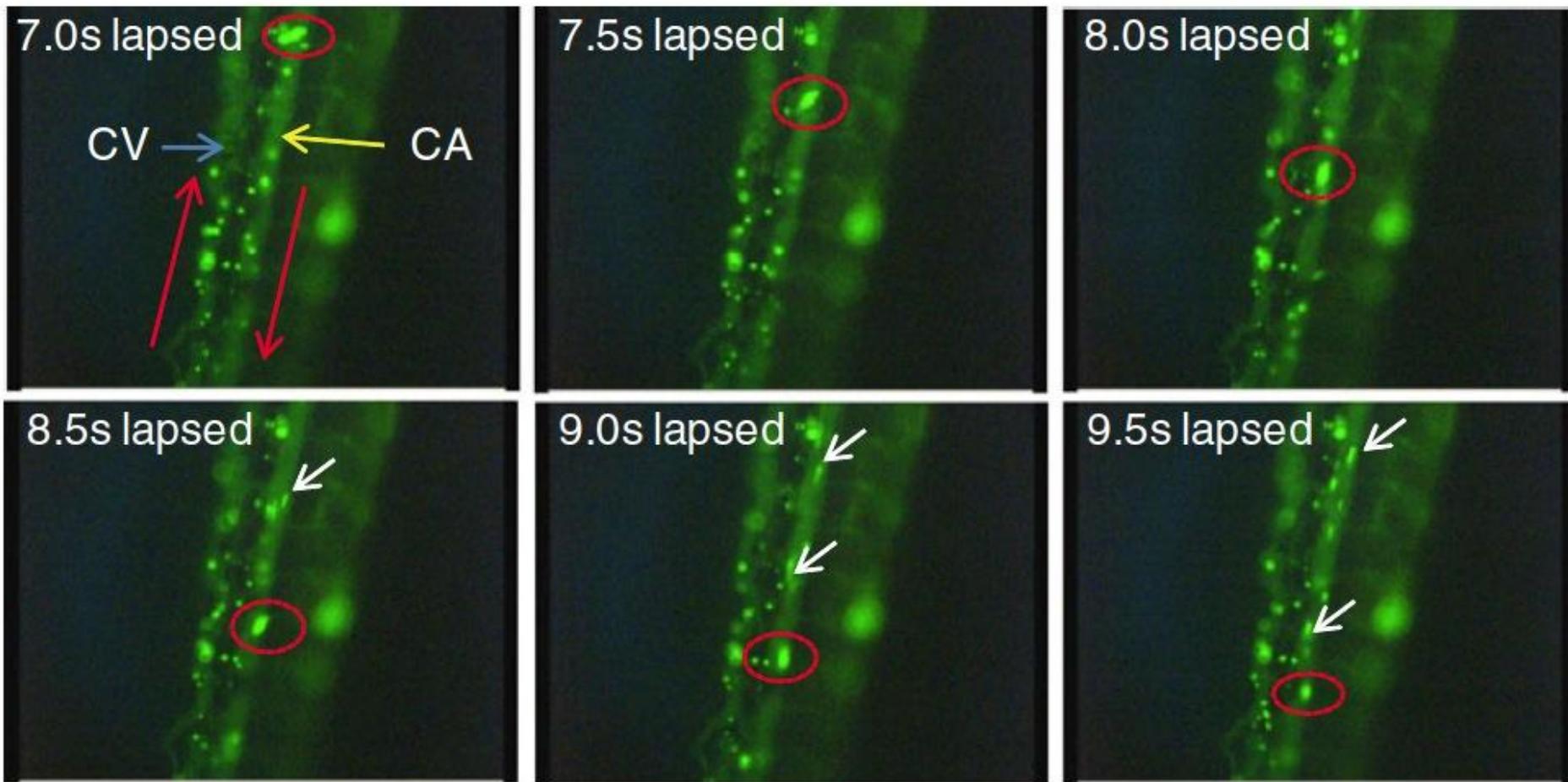


- incubated in FSL-FLRO4 for 2.5d
- present 5 days post wash

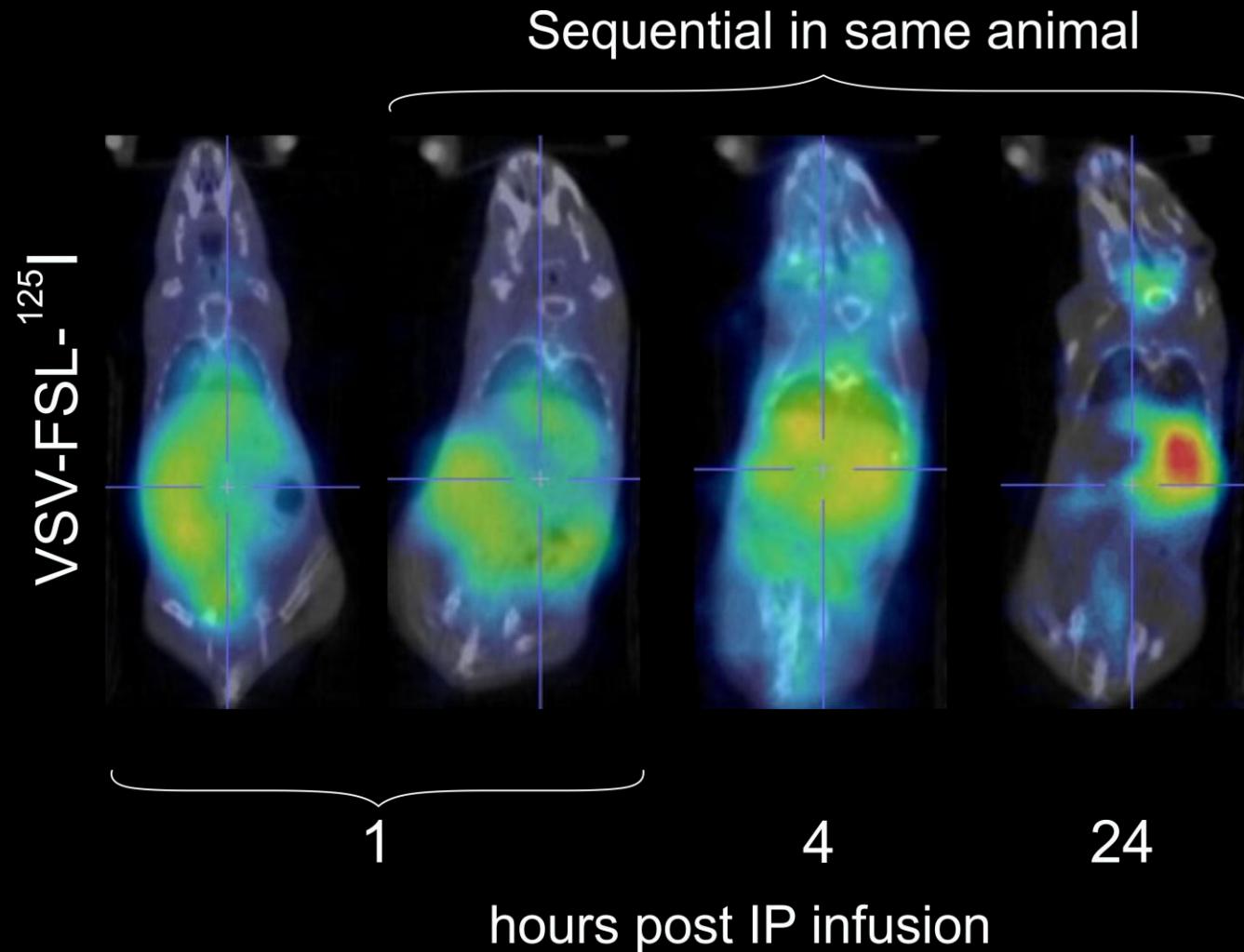
0.5 mg/ml FSL-FLRO4

# *In vivo* kodecyte tracking

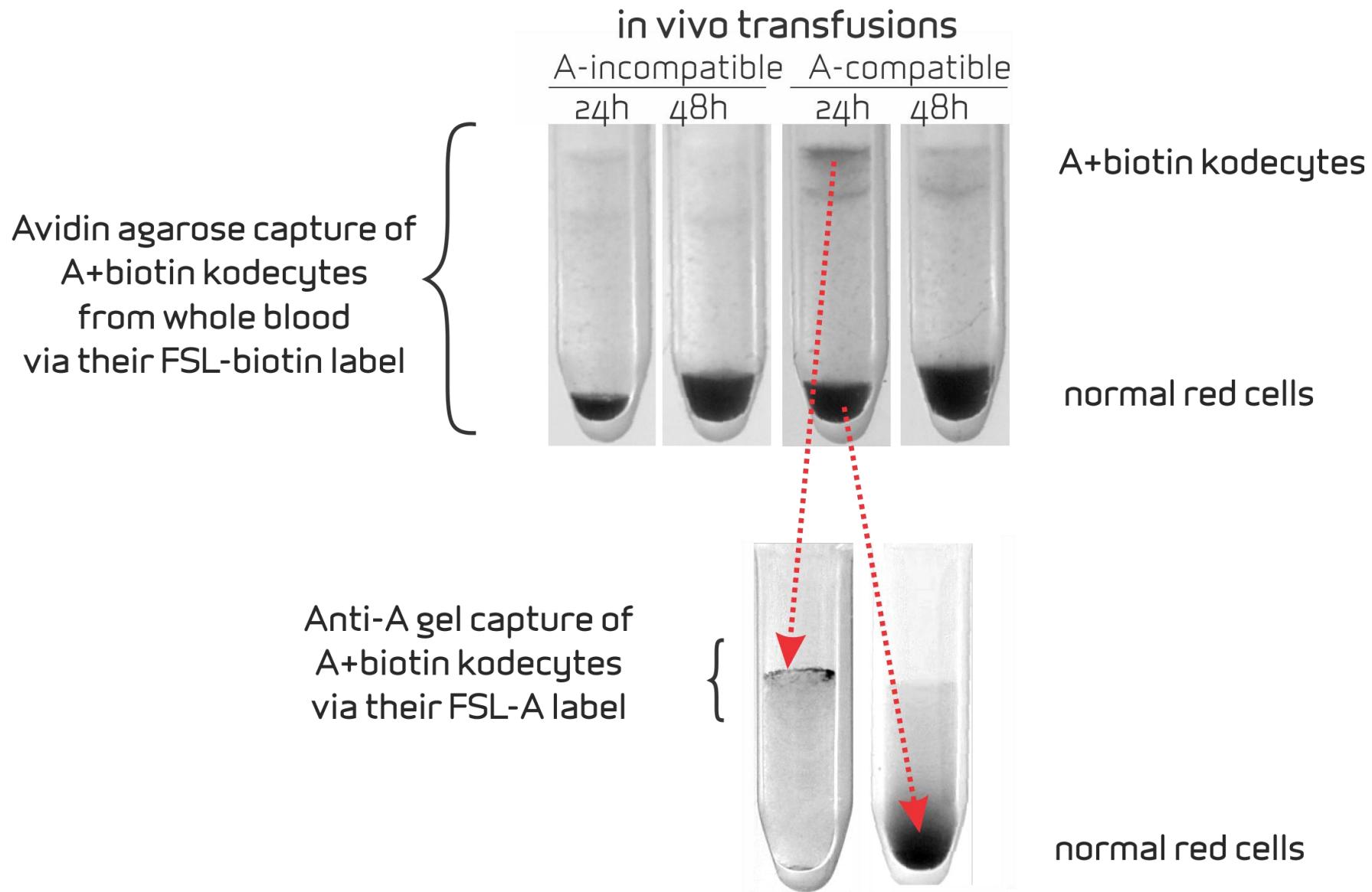
FLRO4 bone marrow kodecytes in Zebra fish

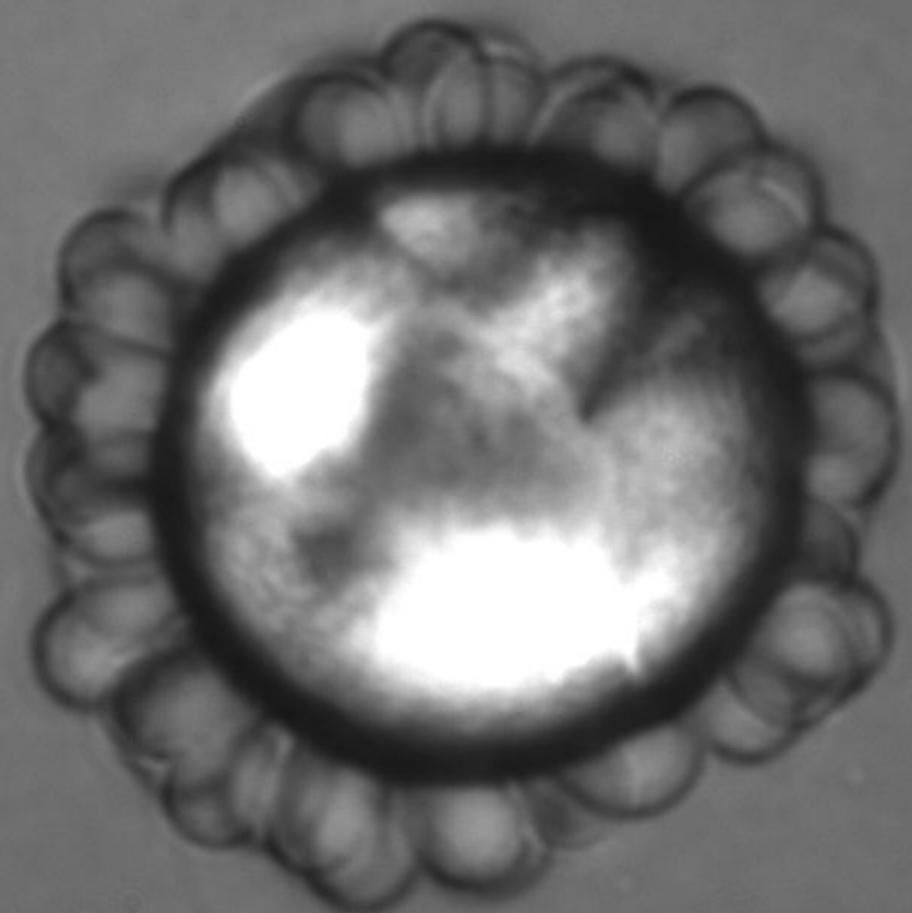


# $^{125}\text{I}$ kodevirions bioimaging

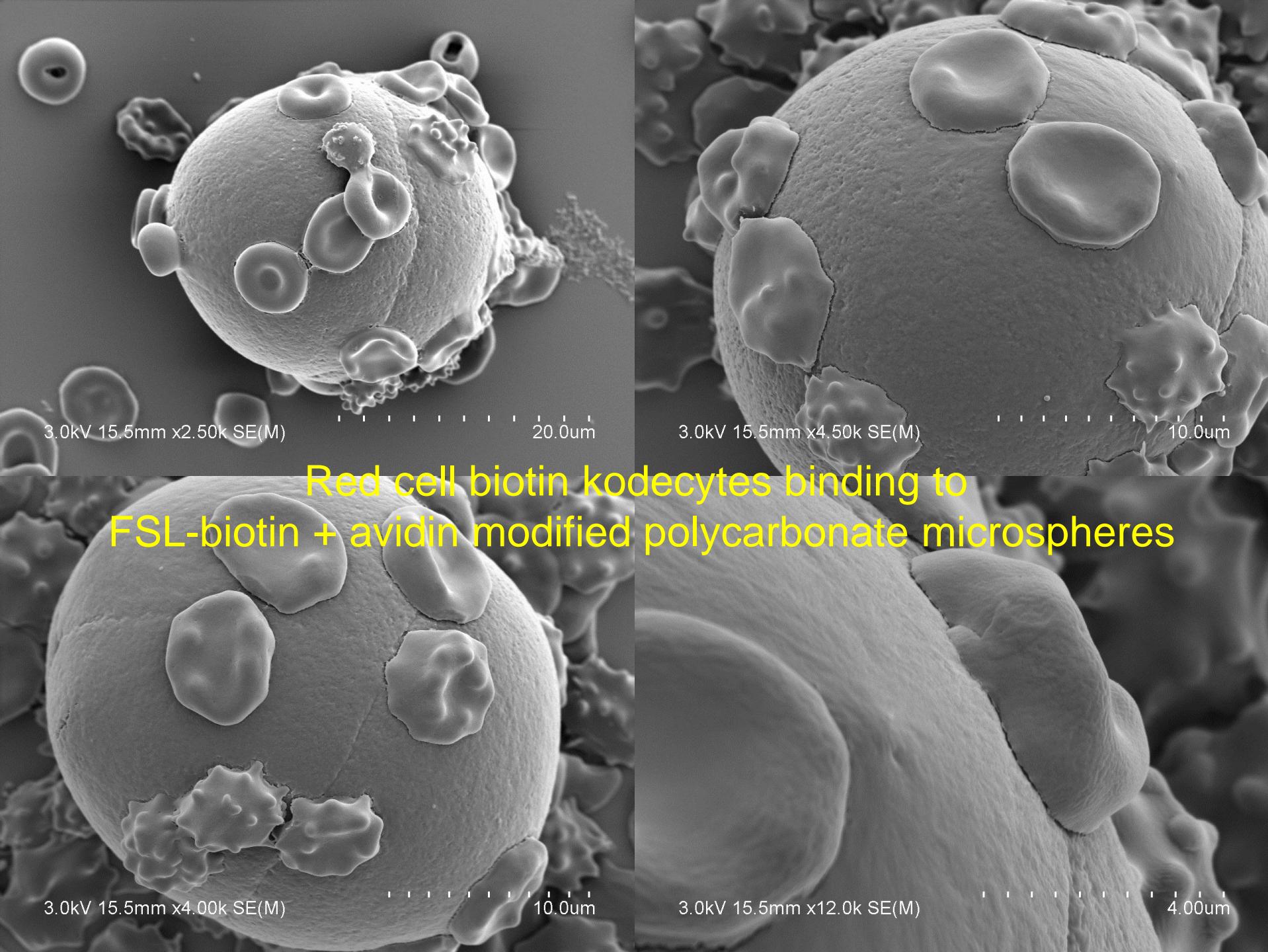


## Recovery of A+biotin kodecytes from whole blood





Red cell biotin kodecytes binding to  
FSL-biotin + avidin modified polycarbonate microspheres





FSL biotin modified *Staphylococcus aureus* binding to

bioactive



difficult or impossible

limited control



FSL-bioactive



very easy

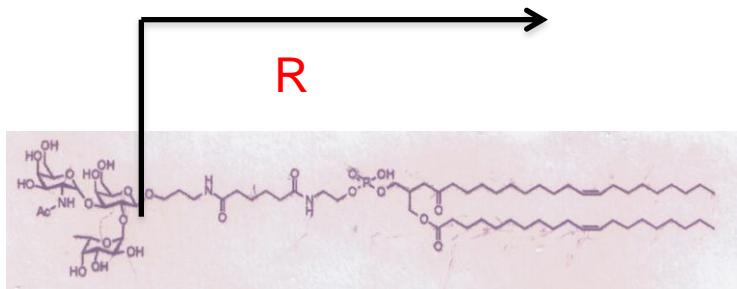
controllable



# Inkjet printing FSL constructs on to paper



# Mapping ABO MAbs with printed FSLs



$\text{A}_{\text{tri}}$  GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-R

A type 1 GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-3GlcNAc $\beta$ -R

## A type 2 GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-4GlcNAc $\beta$ -R

## A type 3 GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-3GalNAc $\alpha$ -R

A type 4 GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-3GalNAc $\beta$ -R

## A type 6 GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-4Glc $\beta$ -R

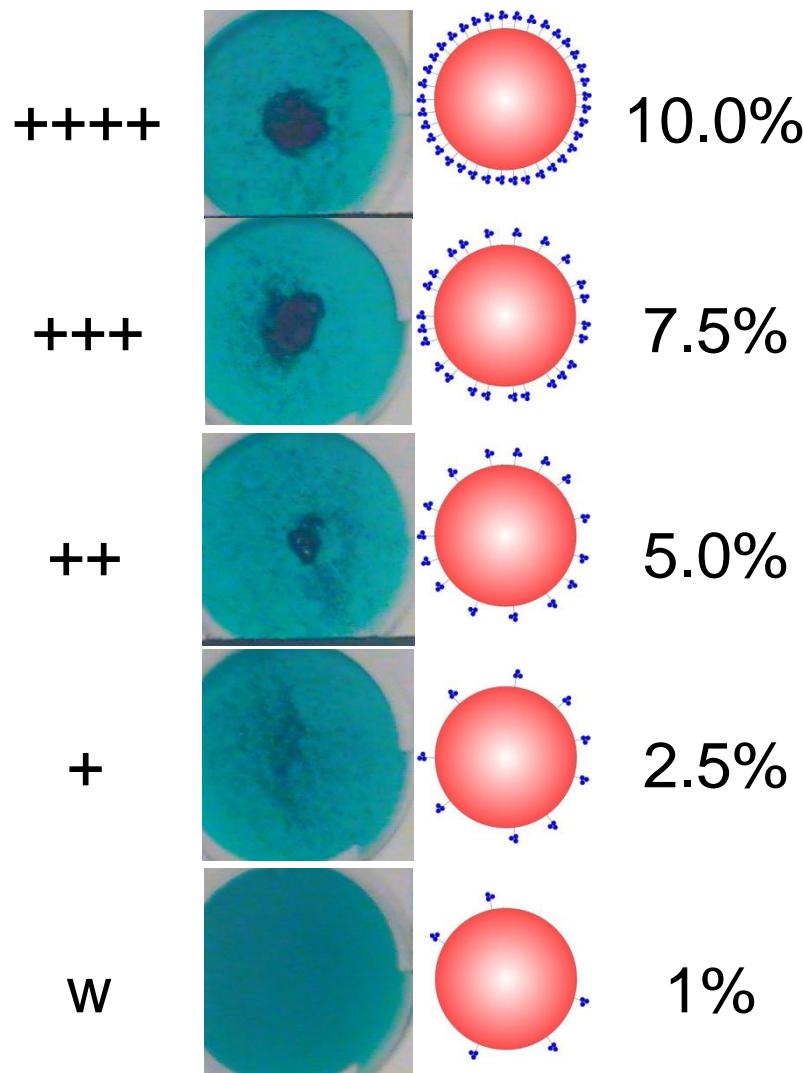
# Established applications

defined synthetic glycolipids    novel glycosylation  
in vivo animal modeling    interactions    biotinylation  
antigen addition    separation  
targeting    fluorescence    neutralisation  
bio-imaging    masking    recovery     $^{125}\text{I}$  radiolabels  
inhibition    bioprinting    anchoring  
bioassays    immobilisation    vitality assays

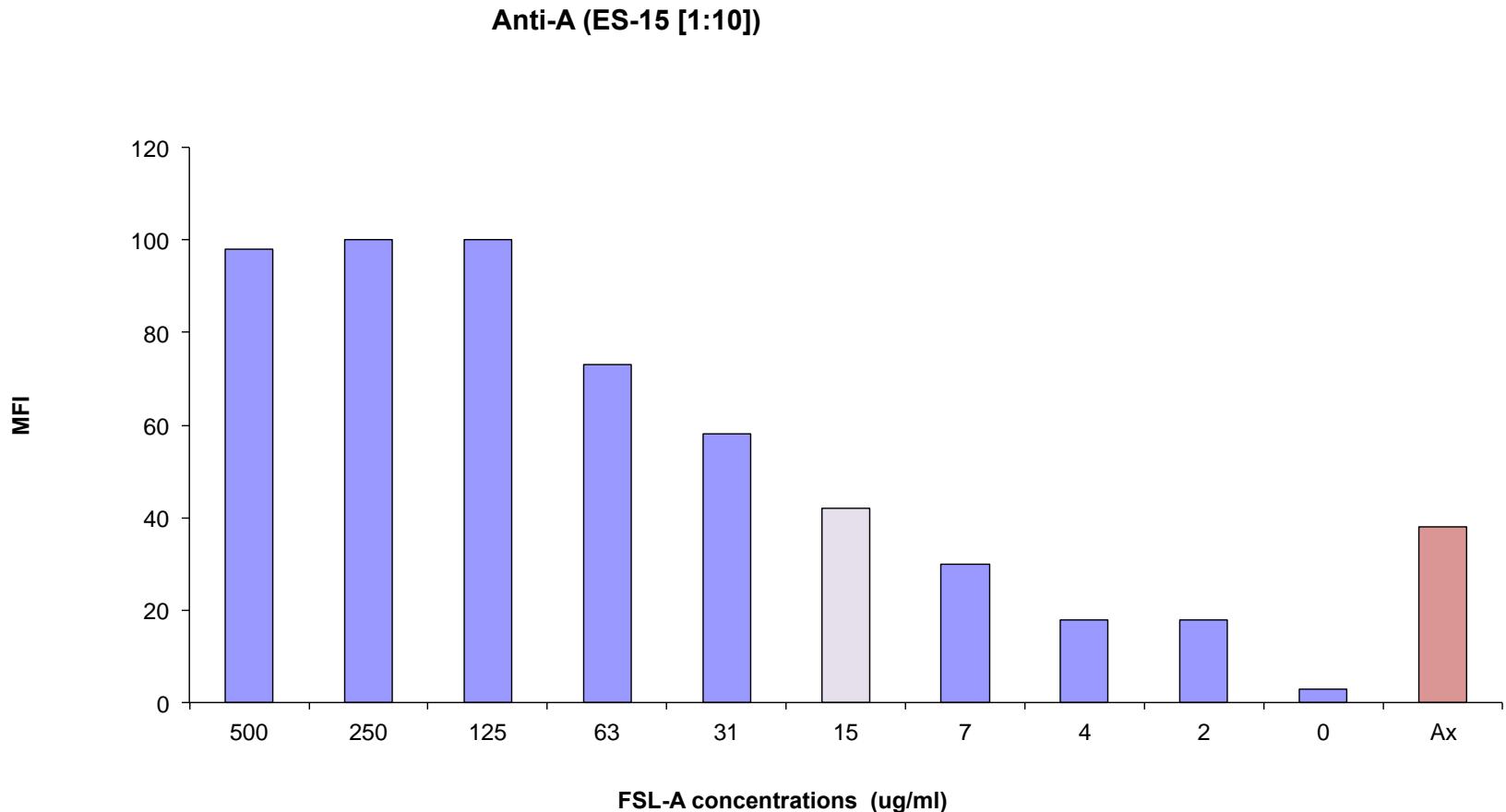
# Serology



# Agglutination - antigen dilutions



# Flow cytometry – A<sub>tri</sub> kodecytes



# AB kodecyte vs different MAbs

MAb serologic scores against AB kodecytes											
FSL-A (ug/ml)	40	36	32	28	24	20	16	12	8	4	0
FSL-B (ug/ml)	300	270	240	210	180	150	120	90	60	30	0
% of 40:300 mix	100	90	80	70	60	50	40	30	20	10	0
Anti-A (MAb-2A)	++++	+++	++	++	++	+	(+)	(+)	-	-	-
Anti-A (MAb-CA1)	+++	+++	+++	++	++	++	++	++	+		

# FSL-tri vs FSL-tetra

A<sub>tri</sub>

GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-**R**

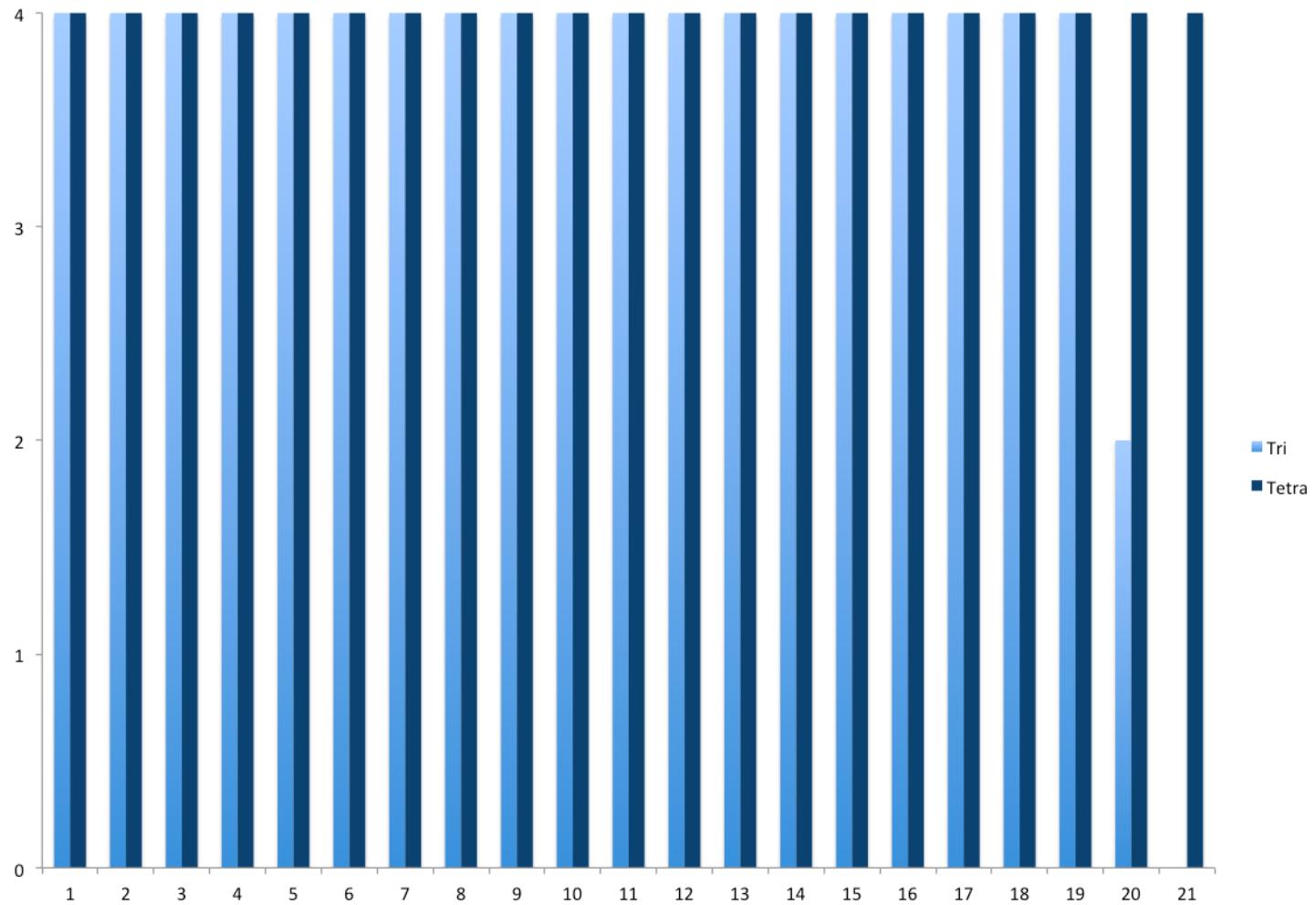
A type 2

GalNAc $\alpha$ 1-3[Fuc $\alpha$ 1-2]Gal $\beta$ 1-**4**GlcNAc $\beta$ -**R**

**R** = spacer - lipid

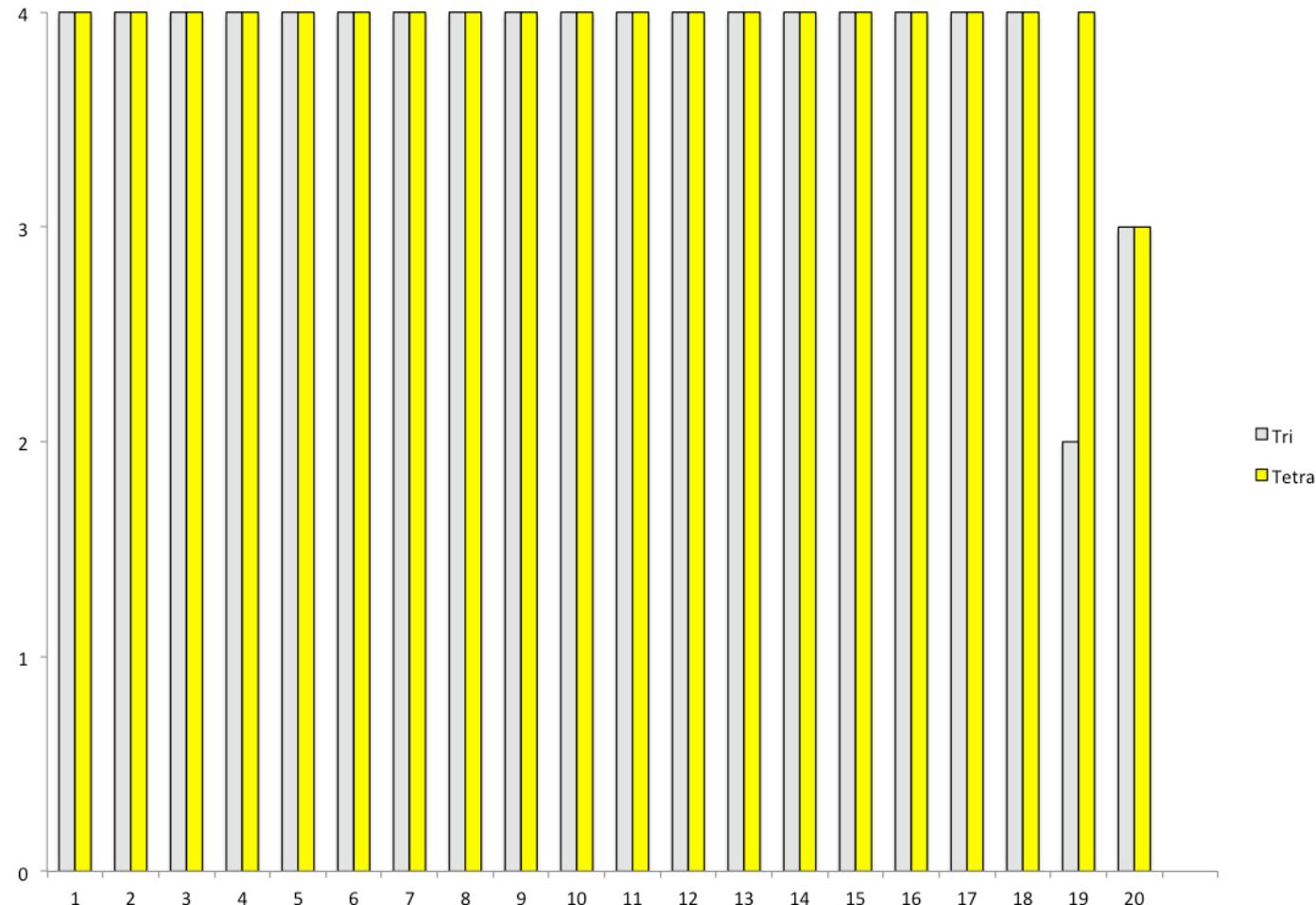
# MAbs vs tri- and tetra- saccharides

## SPECIFICITY (50 µMol)



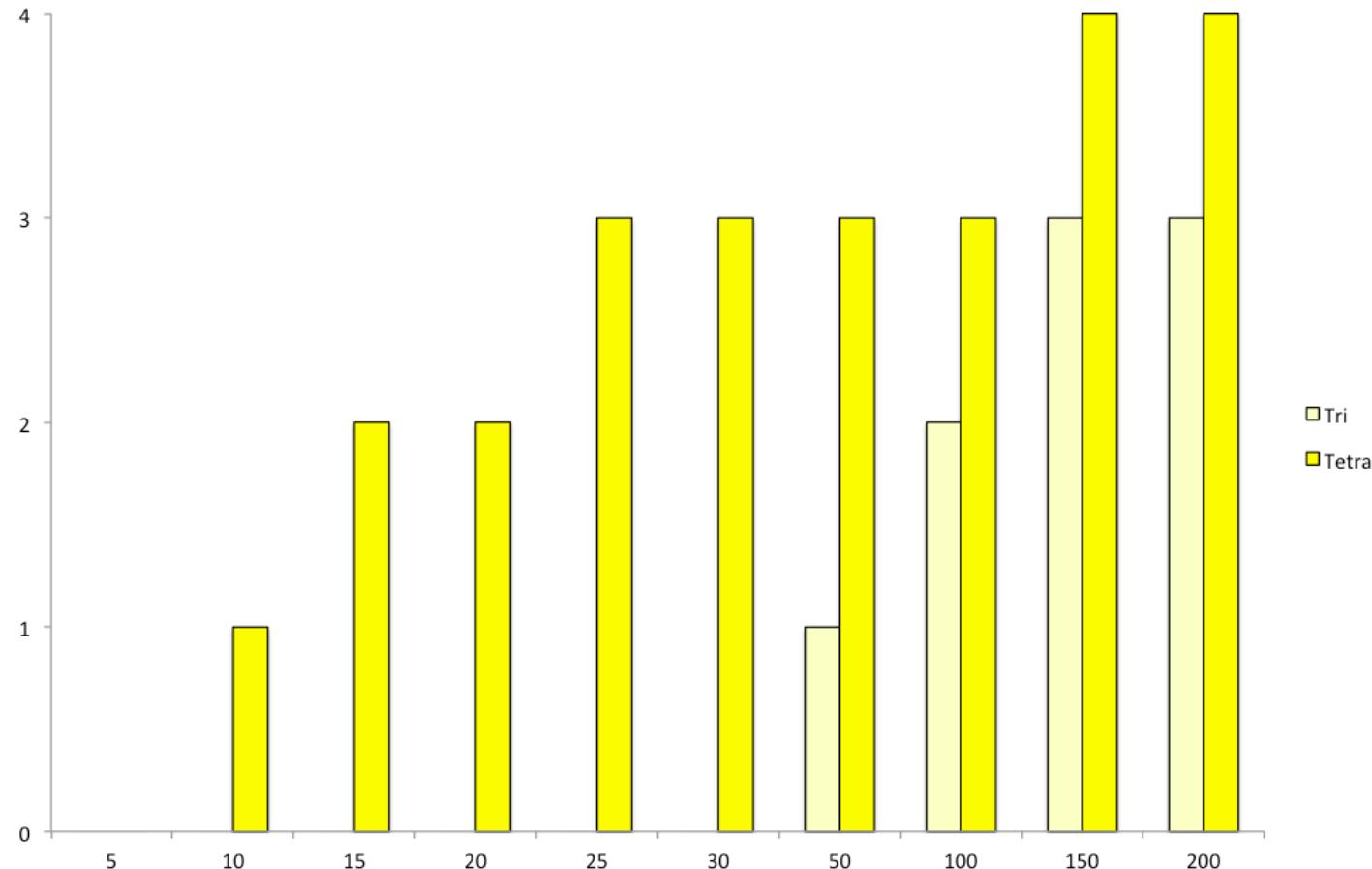
# MAbs vs tri- and tetra- saccharides

## SPECIFICITY (50 µMol)



# MAbs vs tri- and tetra- saccharides

typical µMol SENSITIVITY against poorly tri- reactive anti-B reagents



# Lewis kodecytes vs MAbs

# Lewis kodecytes vs MAbs

# Impossible phenotypes – AcqB kodecytes

A            Gal $\text{NAc}\alpha 1\text{-}3[\text{Fuc}\alpha 1\text{-}2]\text{Gal}\beta 1\text{-R}$

B            Gal         $\alpha 1\text{-}3[\text{Fuc}\alpha 1\text{-}2]\text{Gal}\beta 1\text{-R}$

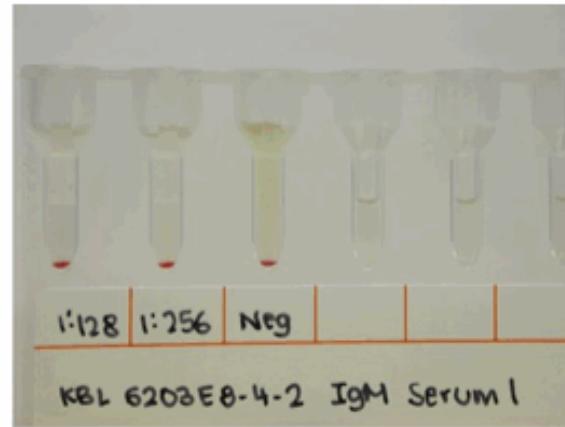
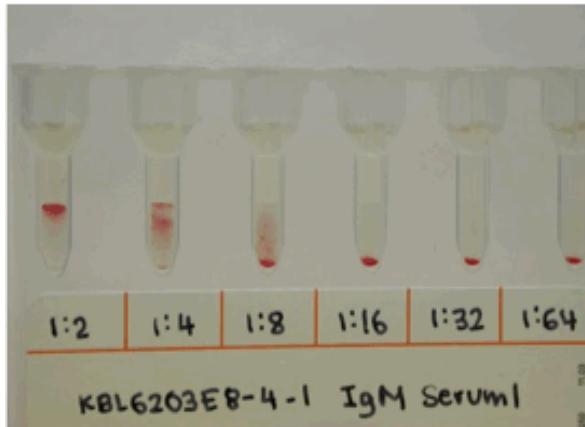
AcqB      GalN     $\alpha 1\text{-}3[\text{Fuc}\alpha 1\text{-}2]\text{Gal}\beta 1\text{-R}$

RBC	Anti-A	Anti-B	Anti-AB		Anti-AcqB	
	6A	6B	8B*	6AB	7AB	AcqB
AcqB-kodecyte	0	0	4	0	4	3
Acq-B	4	0	4	4	4	4

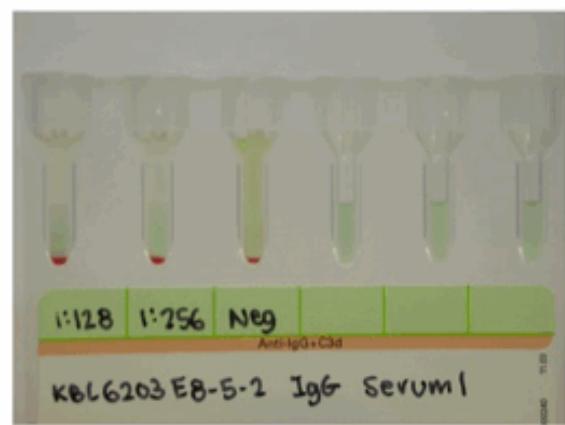
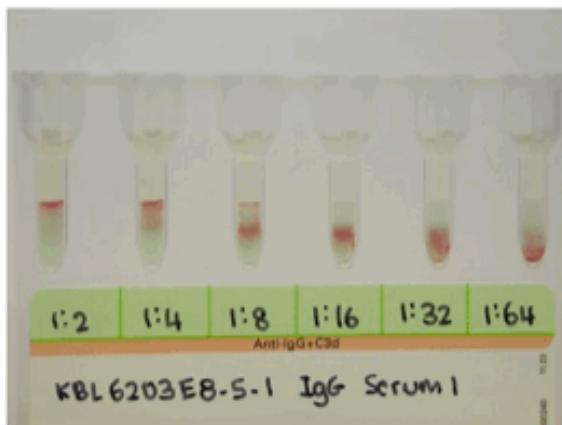
\* anti-B 8B also reacts with acquired B

# Antibody quantitating kodecytes

Galili kodecytes vs pig to human (xenotransplant) serum



Serum 1



Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-R

# KODE™ technology QC systems

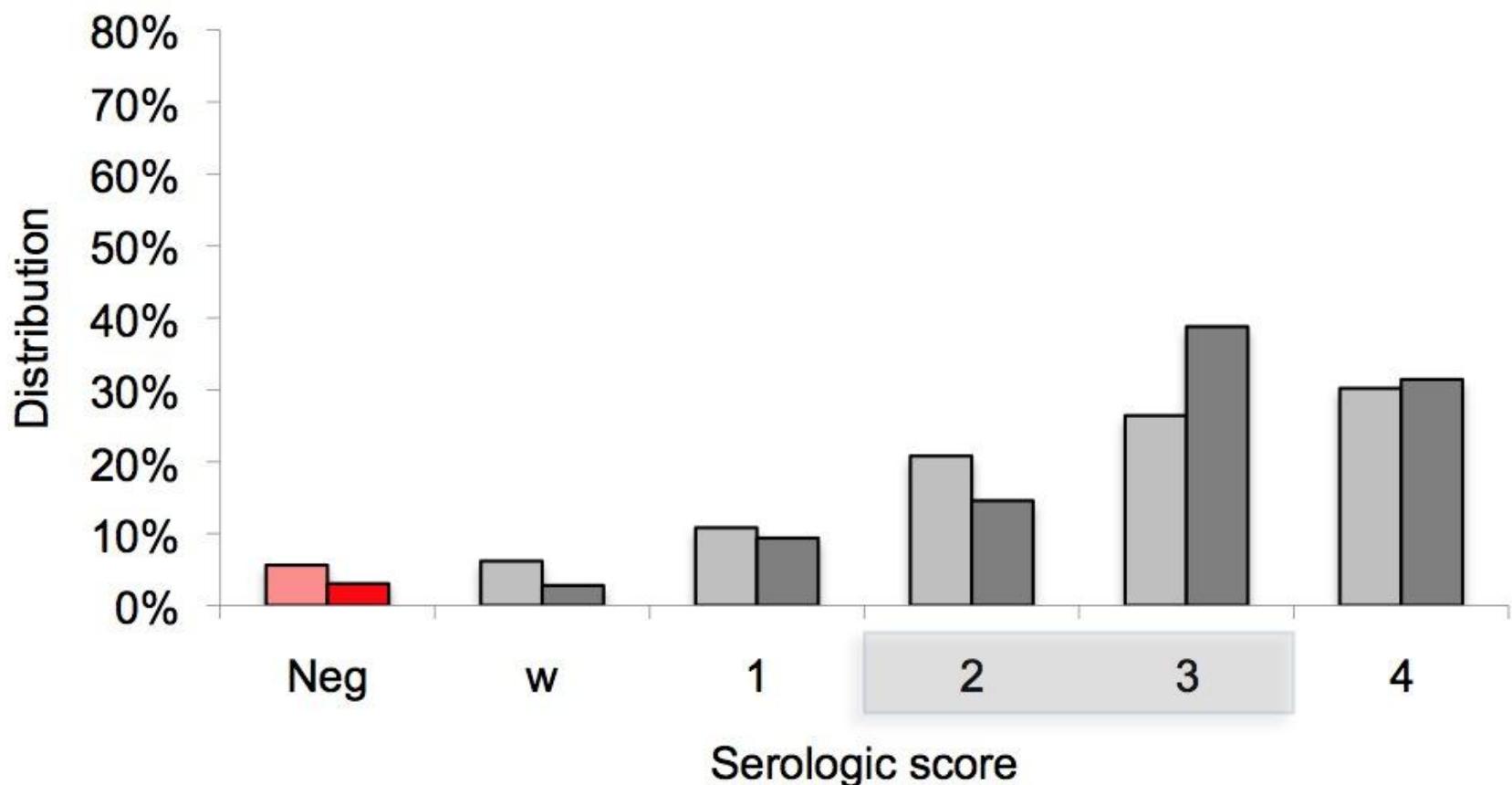


# Kodecyte sensitivity to MAb deterioration

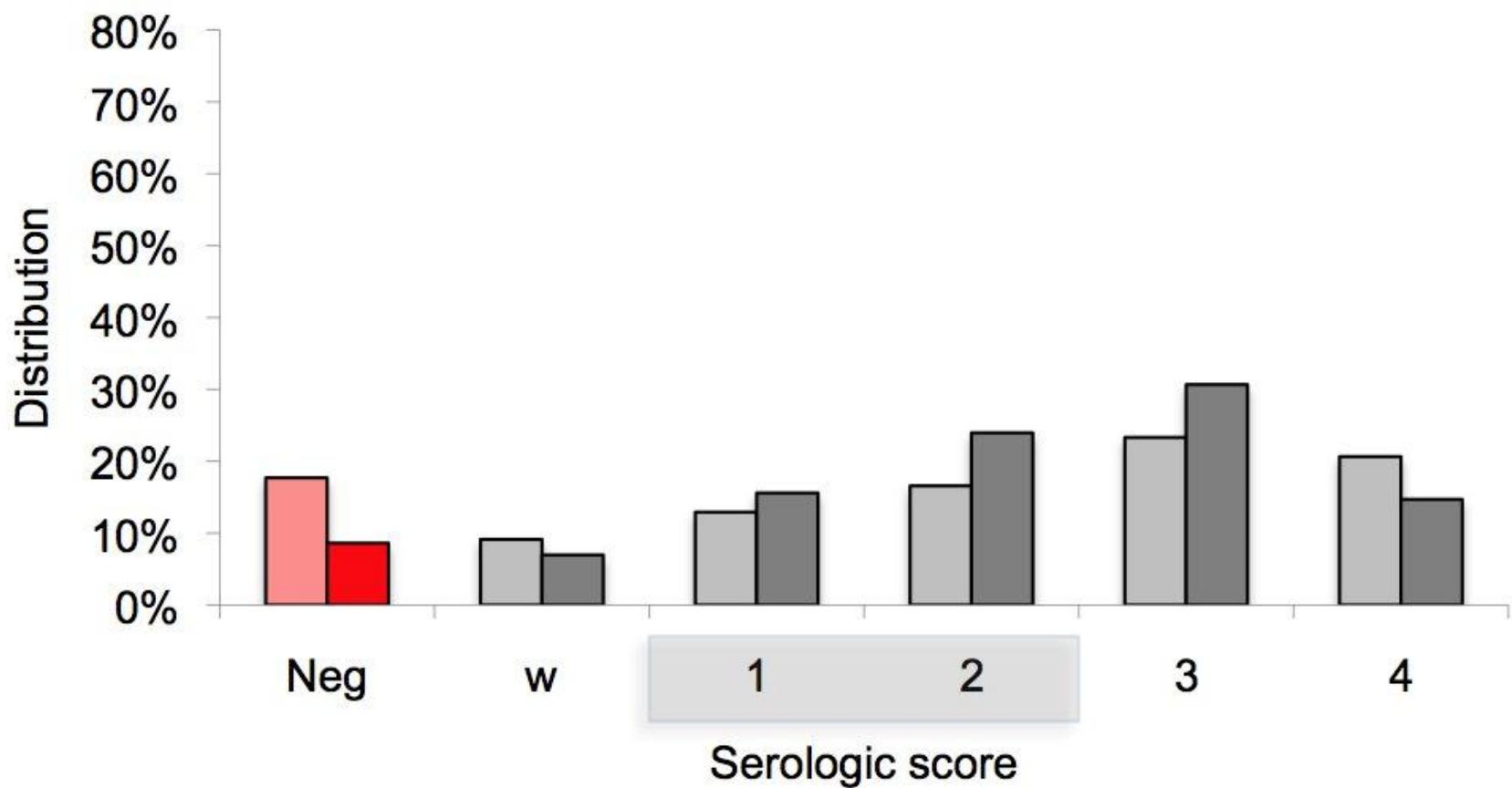
	anti-A degradation			
Time (mins @ 60°C)	0	10	12.5	15
Titre vs A <sub>2</sub> cells	<b>1:256</b>	<b>1:32</b>	<b>1:8</b>	<b>1:4</b>
% remaining activity	100%	13%	3%	1.5%

	Serology against degraded anti-A			
Natural A <sub>2</sub> cell	++++	++++	++++	++++
A kodecyte 20ug/ml	++++	++	-	-
A kodecyte 15ug/ml	+++	+	-	-

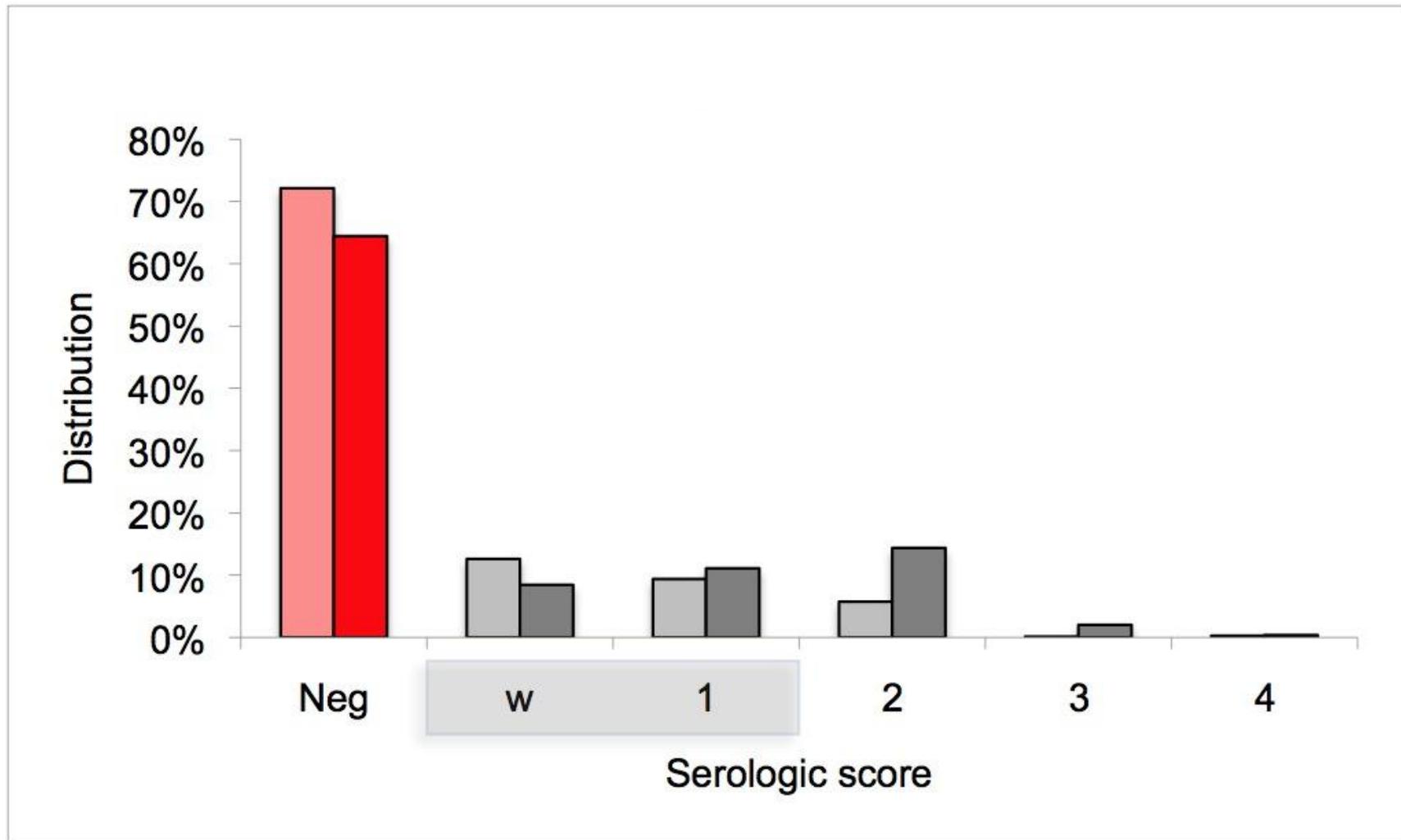
# kodocytes (grade 2+ or 3+)

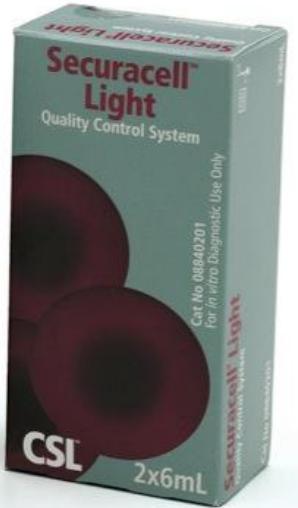


# kodocytes (grade 1+ or 2+)



# kodocytes (grade w or 1+)

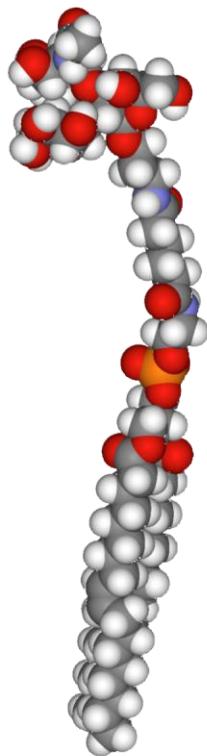




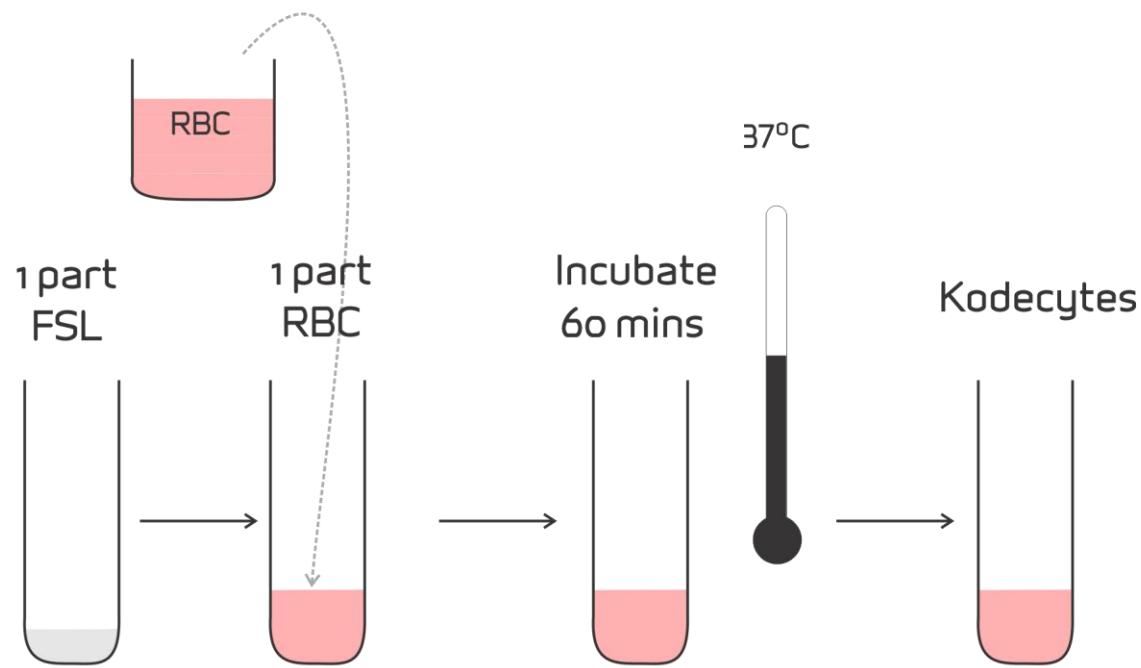
# KODE™ technology teaching



# Preparation of kodecytes



Function  
Spacer  
Lipid



O cells were transformed into AB cells with controlled but varied levels of A and B antigen

# Examples of typical anti-A grades for natural cells & kodecytes

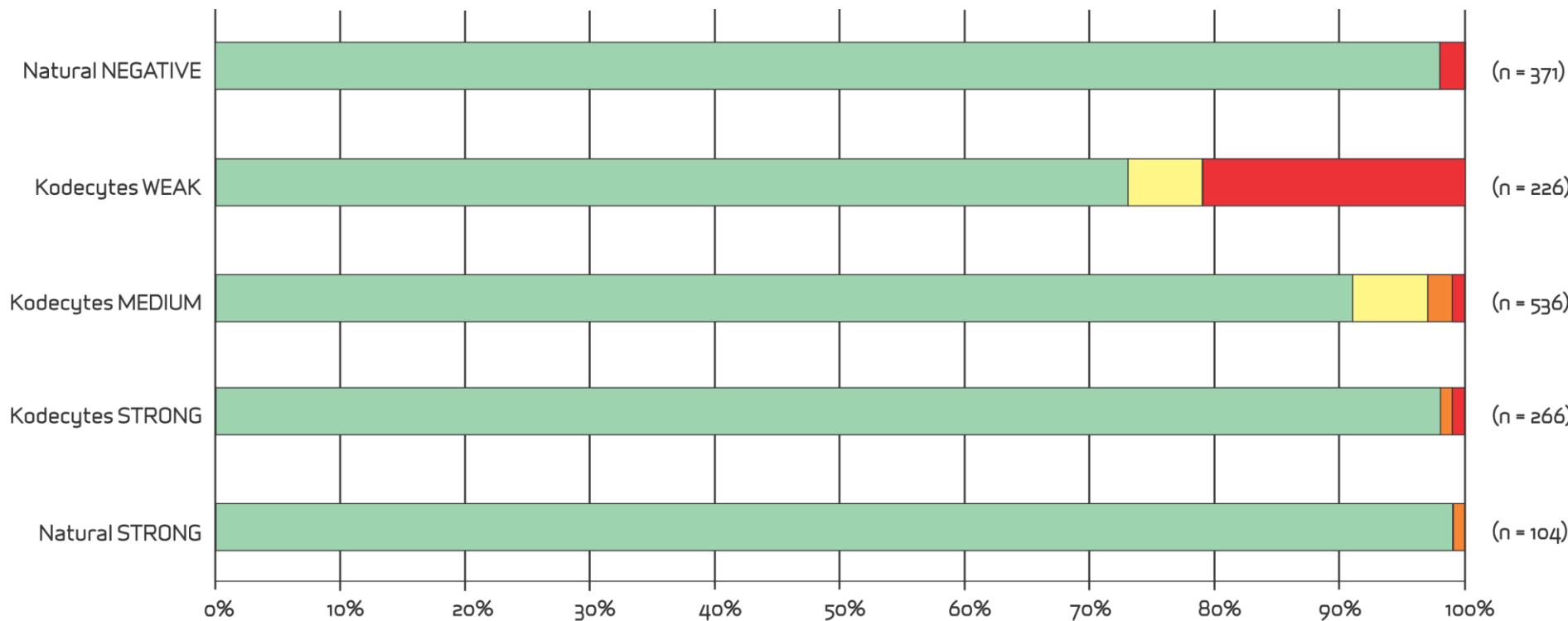
Grade	++++	++++	+++	++	+	-	-
Kodecyte	A100 B300	A 50 B150	A25 B75	A12 B38	A 6 B19	A3 B9	A1.5 B4

Grade	-	++++	++++	++++	-		
Natural cell	O	A <sub>1</sub> B	A <sub>1</sub> B	A <sub>2</sub>	B		

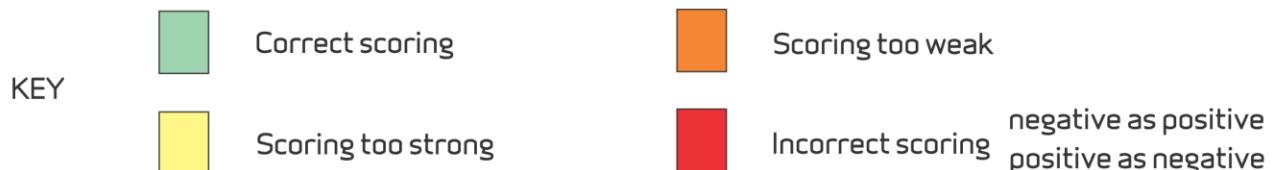
# Examples of typical teaching panels with natural cells & kodecytes interspersed

Grade	-	++++	++++	-	-	-	++++	-	+	-	++	+
Panel cell	O	A <sub>2</sub>	A100 B300	B	O	A6 B19	A <sub>2</sub>	A6 B19	A12 B38	A6 B19	A12 B38	A12 B38

Grade	-	++	++	-	+	-	+++	+++	+++	+++	+++	+++
Panel cell	O	A12 B38	A12 B38	B	A12 B38	B	A25 B75	A <sub>2</sub>	A25 B75	A <sub>2</sub>	A25 B75	A <sub>2</sub>



### Analysis of the accuracy of student results



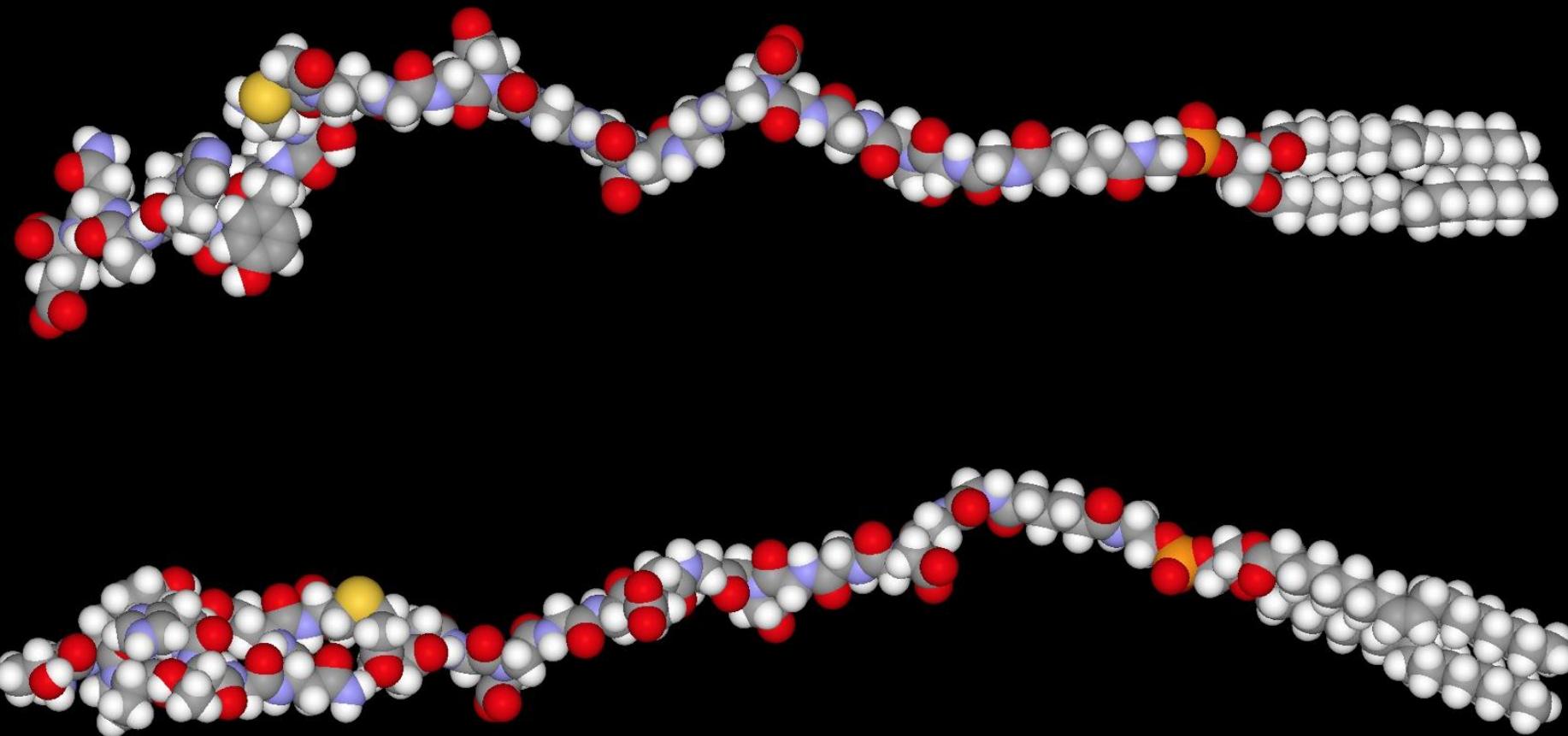
# New tools in development ...

- Hemolysis panels
- Indirect antiglobulin
- Polyagglutination/panagglutinin
- AIHA – cold & warm
- LFA/HFA

# KODE™ technology diagnostics



# MUT & Mur vMNS FSL constructs



# MUT, Mur & MUT+Mur kodescytes

Heathcote et al., Transfusion 2010; 50: 635-641

Red Cell	Characterized Polyclonal Sera		Monoclonal Reagents
	Anti-MUT (n=4)	Anti-Mur (n=5)	Anti-Mi <sup>a</sup> /Mi III/Mur (n=4)
MUT kodescyte	4/4	0/5	0/4
Mur kodescyte	0/4	5/5	0/4
<b>MUT+Mur kodescyte</b>	<b>4/4</b>	<b>5/5</b>	<b>0/4</b>
Gp.Mur (n=6)	4/4	5/5	4/4



# MUT+Mur kodecyte performance

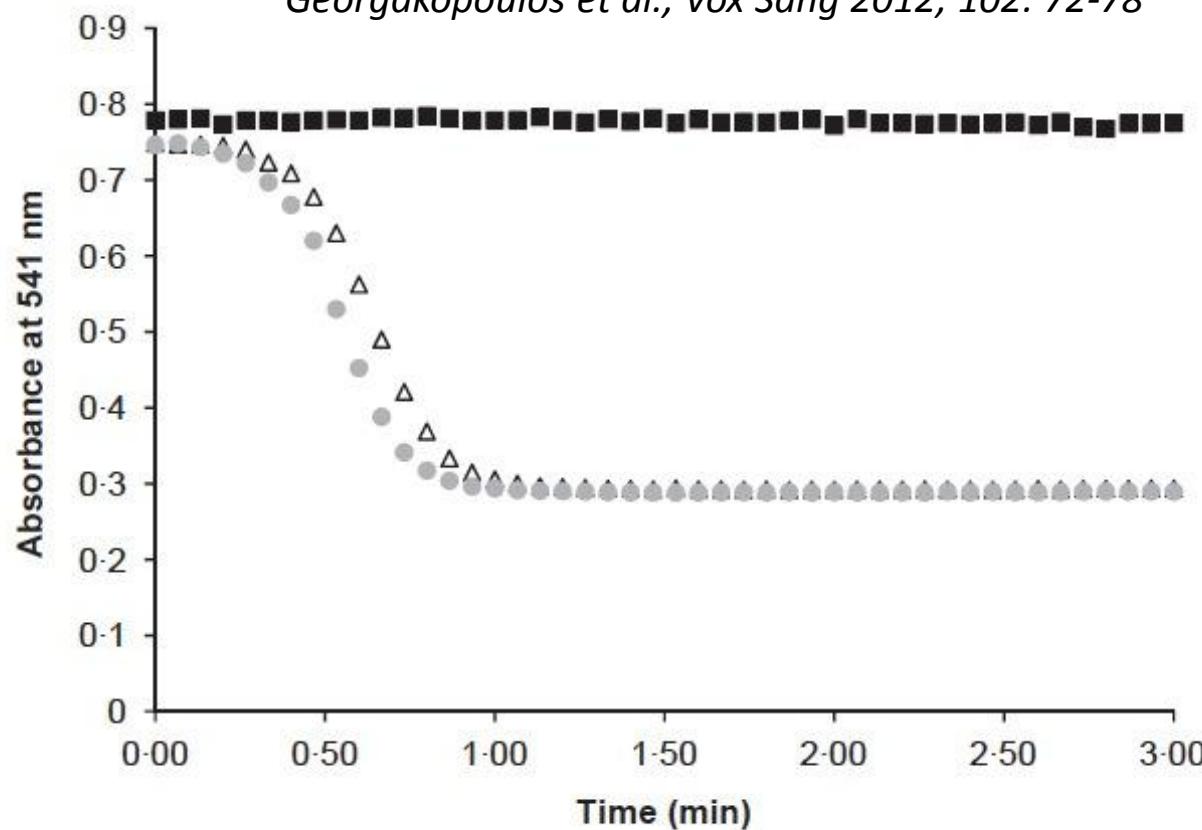
Nadarajan et al., Vox Sang 2011: early view

	Screening Panel		
Antibody	Standard +/- Mi <sup>a</sup>	vMNS kodecytes	Kodecyte Impact
Screens (n)	95,587	47,914	
Pos Screen	3.18%	3.97%	
Unresolved	0.47%	0.70%	+0.33%

	Screening Panel		
Antibody ID	Standard +/- Mi <sup>a</sup>	vMNS kodecytes	Kodecyte Impact
vMNS	0.9%	5.8%	+ 4.9%
Le <sup>a</sup>	6.7%	3.2%	- 3.5%
Le <sup>b</sup>	2.5%	1.7%	- 0.8%
P1	0.4%	0.2%	- 0.2%
Others	89.5%	89.1%	

# Cytomegalovirus kocytes

Georgakopoulos et al., Vox Sang 2012; 102: 72-78



**Fig. 3** Haemolysis curves of 0.8 mg/ml cytomegalovirus kocytes following incubation with ■ buffer, △ Intravenous immunoglobulin or ● European Pharmacopoeia Biological Reference Preparation standard and the addition of 153 CH<sub>50</sub>/ml of Guinea pig complement. The samples were analysed in duplicate, and the mean haemolysis curve for each sample is shown.

# Syphilis kodecytes

Table 2. FSL-SYPH3 gelcard Method 2 comparison with TPHA and RPR of 98 EIA+TPPA positive samples

EIA + TPPA validated positive samples	Positives by method		
	RPR	TPHA	Method 2 <b>FSL-SYPH3</b>
n = 98	56	90	96

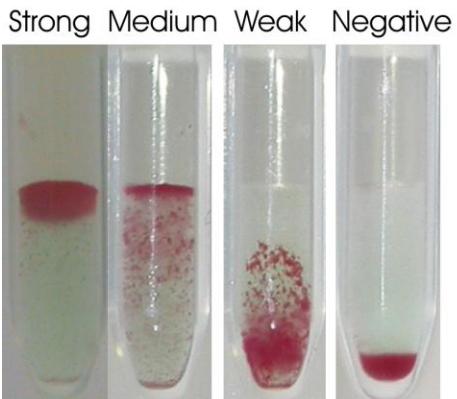
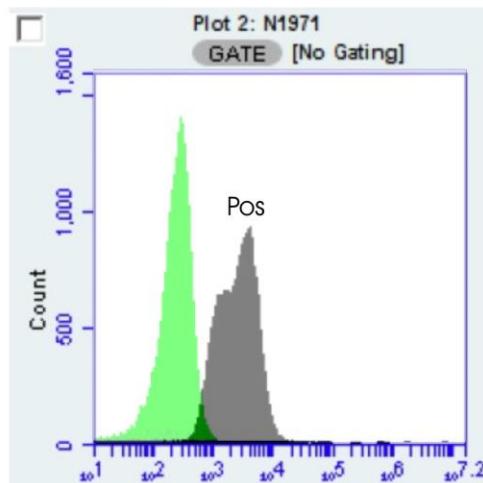
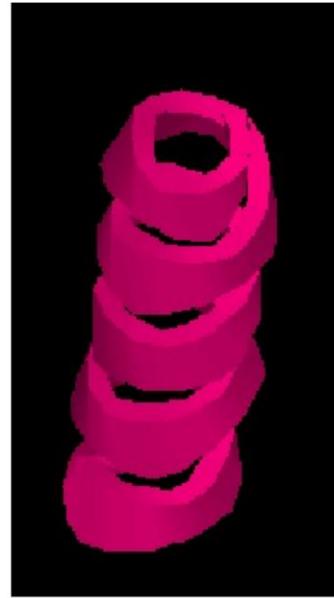
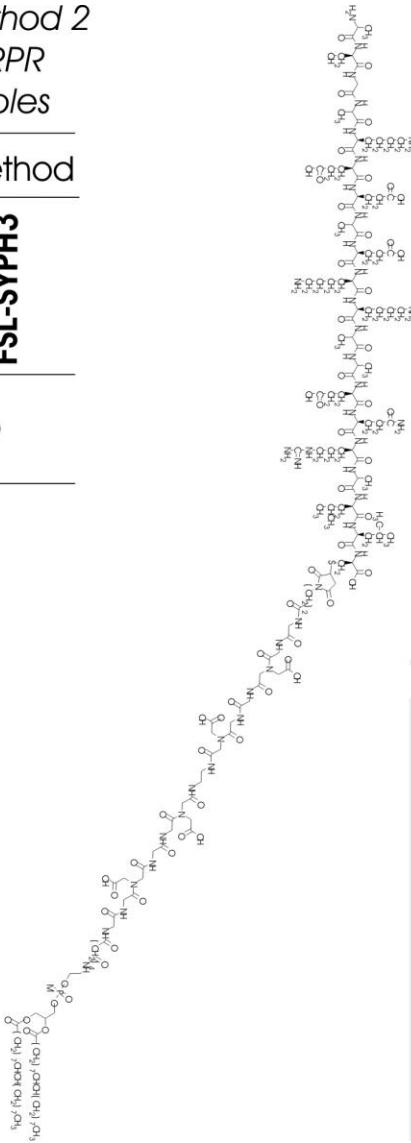


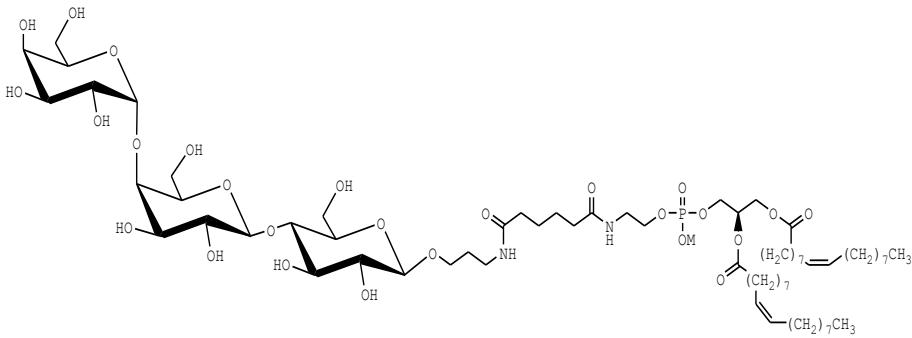
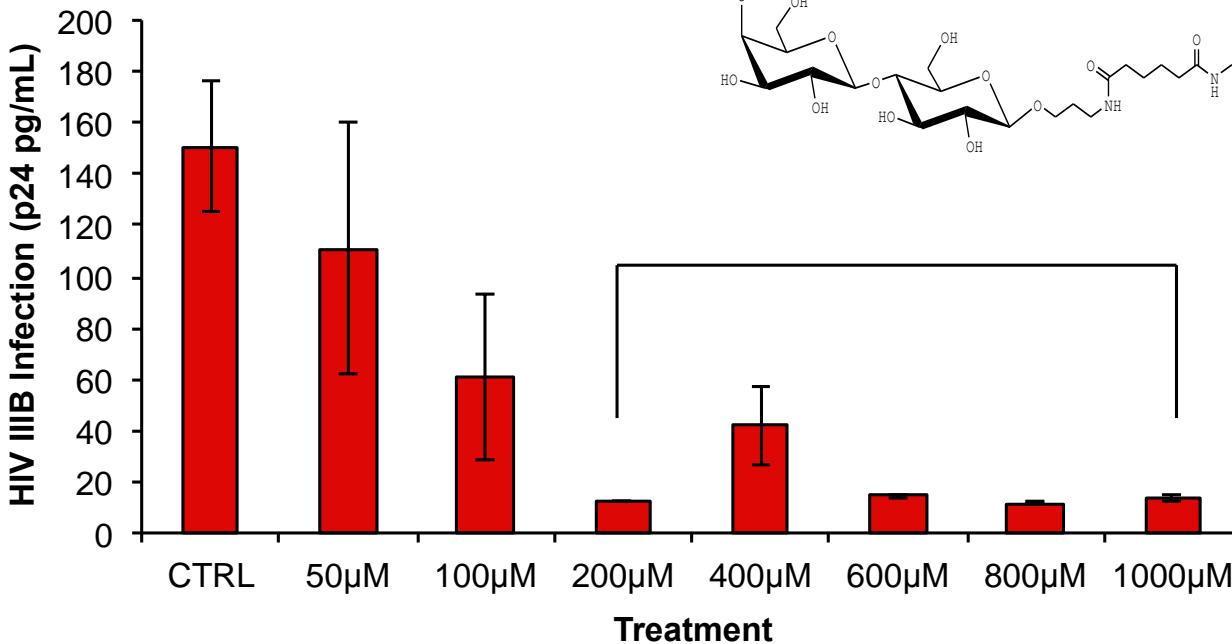
Figure 3. Examples of FSL-SYPH3 kodecyte gelcard reactions



# KODE™ technology therapeutics



# Inhibition of HIV cell infection



**FSL-GB3 Inhibition of X4 HIV-1<sub>III B</sub> Infection of PBMCs**  
(\*p<0.05; 200-1000μM)

# TEMPORARY SUPPRESSION OF LEWIS BLOOD-GROUP ANTIBODIES TO PERMIT INCOMPATIBLE TRANSFUSION

P. L. MOLLISON  
M.D. Cantab., F.R.C.P.

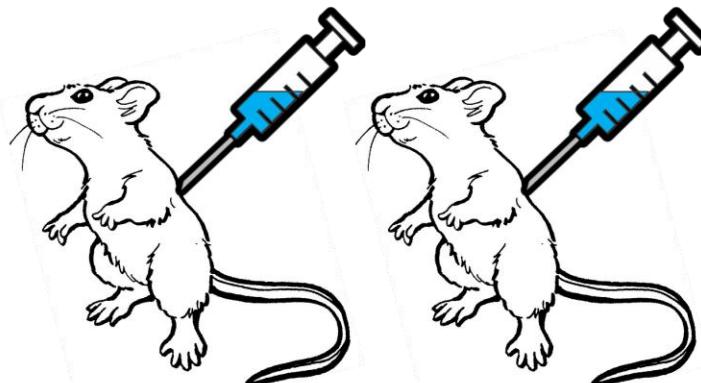
MARGARET J. POLLEY  
B.Sc. Wales

PATRICIA CROME  
M.B. Lond.

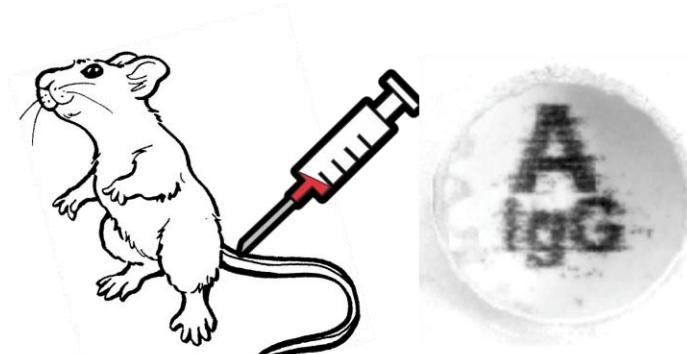
*From the Medical Research Council Experimental Hæmatology  
Research Unit, Wright-Fleming Institute of Microbiology,  
St. Mary's Hospital Medical School, London, W.2*



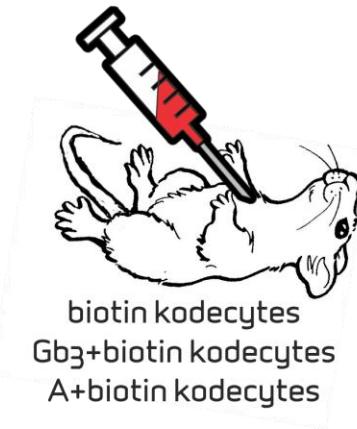
Patrick Loudon Mollison, 1914-2011



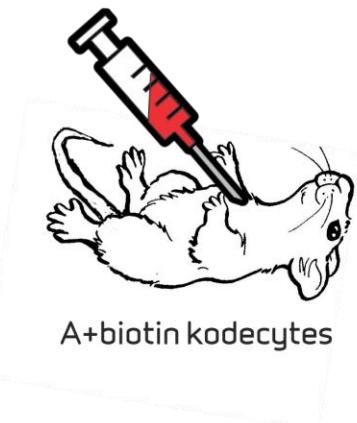
Immunise with A Le(a-b+) saliva



Determine anti-A status



**COMPATIBLE**  
(anti-A negative)

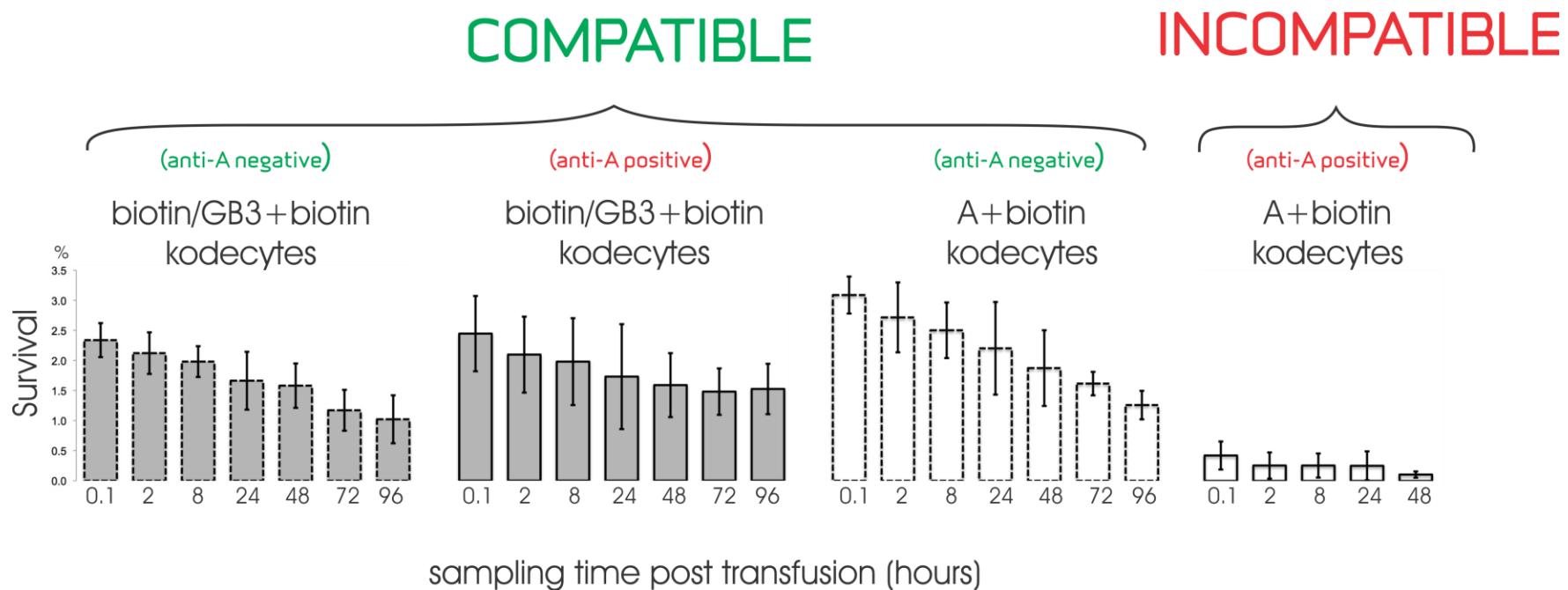


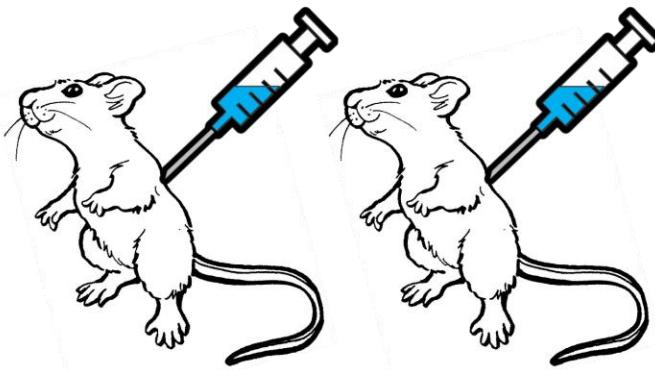
**INCOMPATIBLE**  
(anti-A positive)



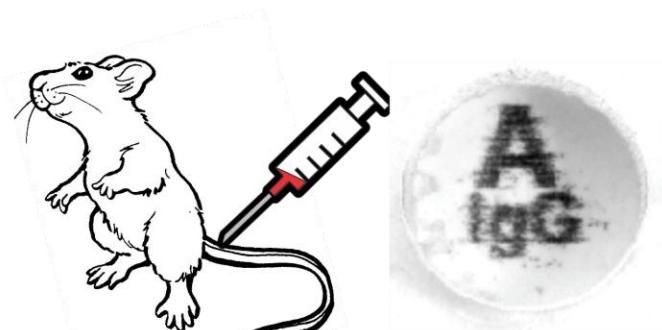
Bleed to determine kodecyte survival

# Survival of 3% compatible & incompatible kodecyte transfusions

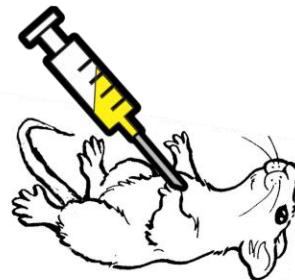




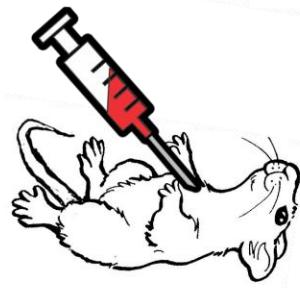
Immunise with A Le(a-b+) saliva



Determine anti-A status - POSITIVE



Infuse FSL-A

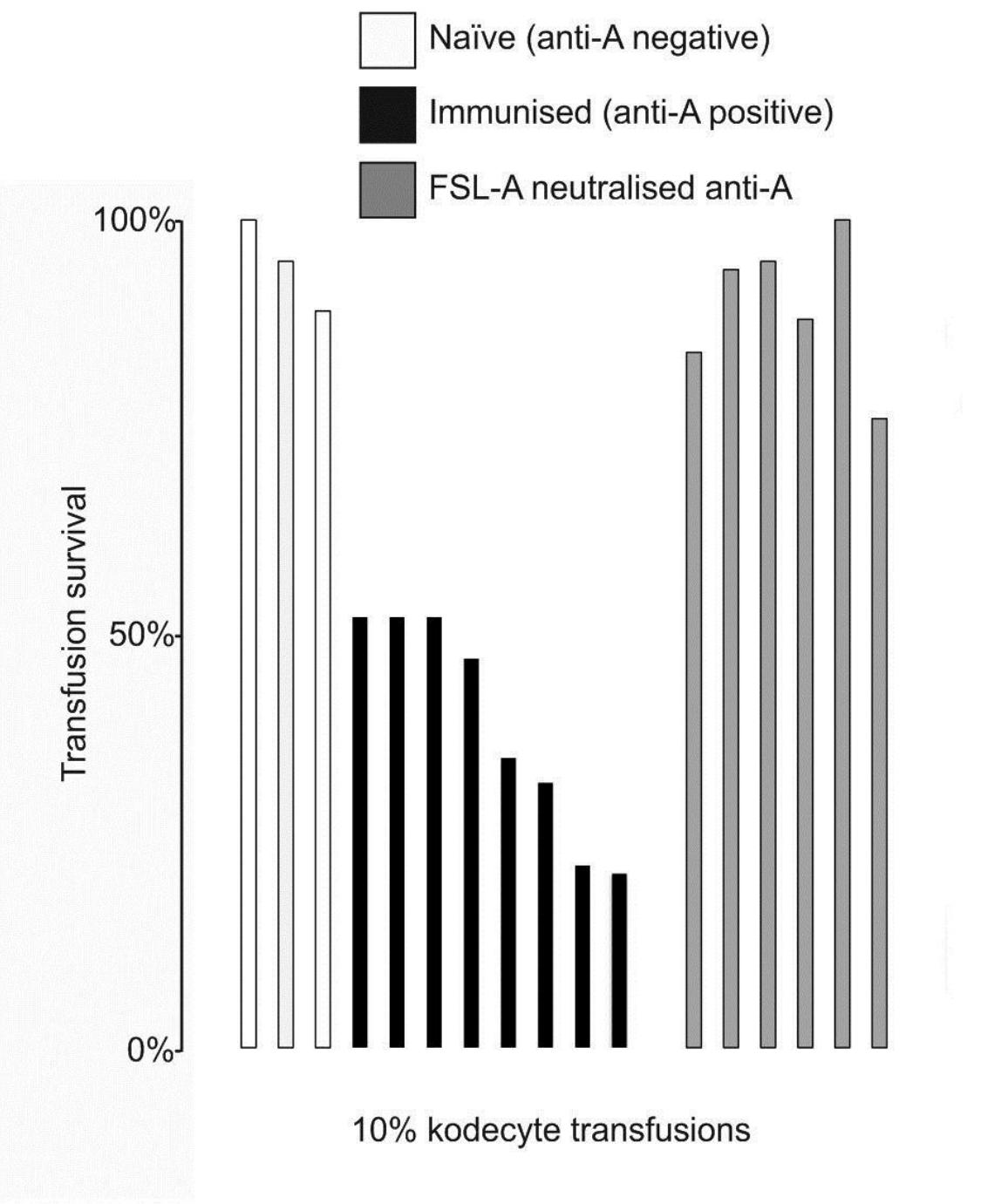


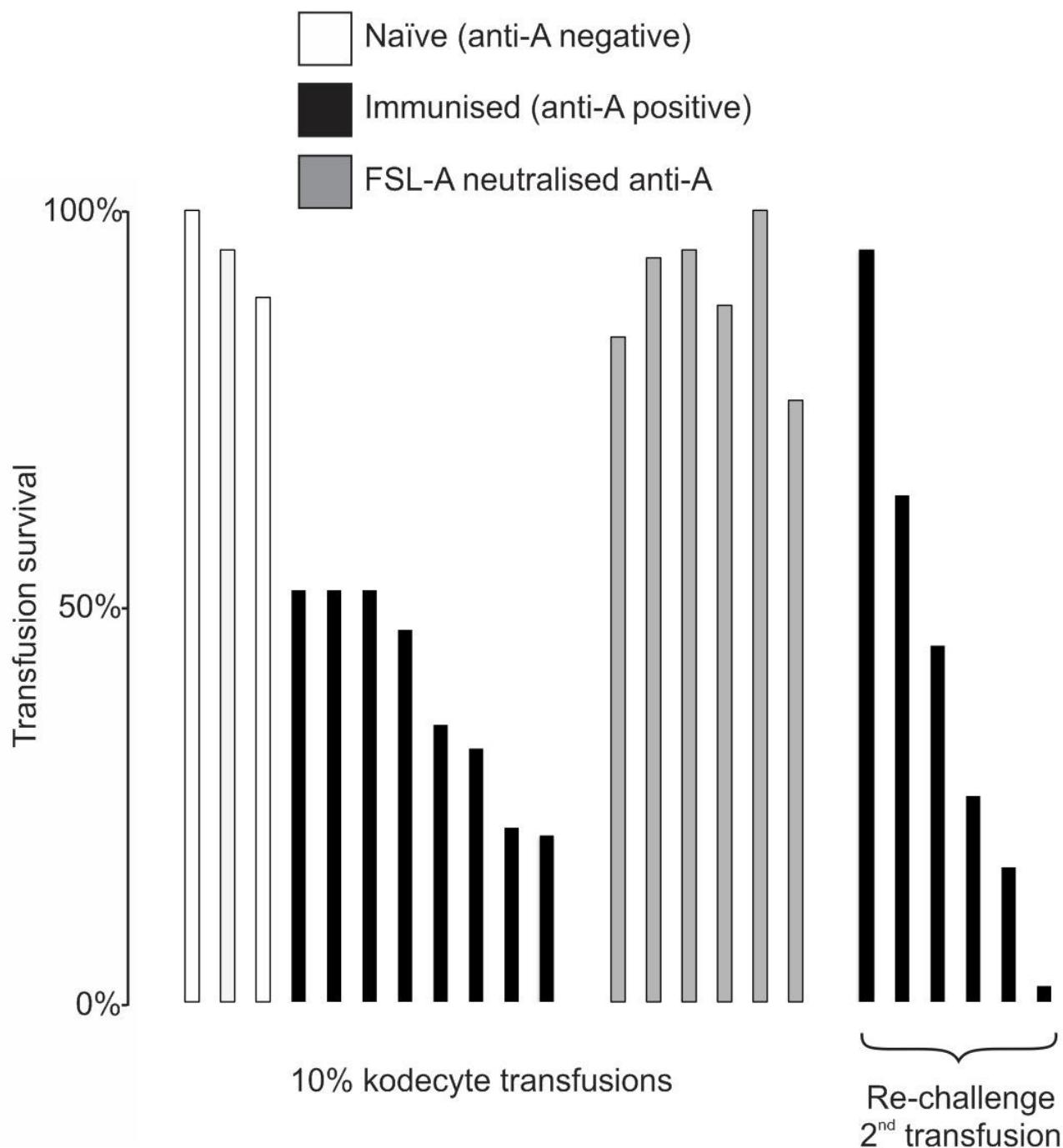
A+biotin kodecytes

**INCOMPATIBLE**  
(anti-A positive)



Bleed to determine kodecyte survival







KODE  
Biotech

biosurface innovation

accelerating R&D and  
creating new product possibilities



# **5<sup>TH</sup>** INTERNATIONAL MONOCLONAL ANTIBODY WORKSHOP

**MARCH 27-30, 2014**

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<http://support.nybloodcenter.org/monoclonal>