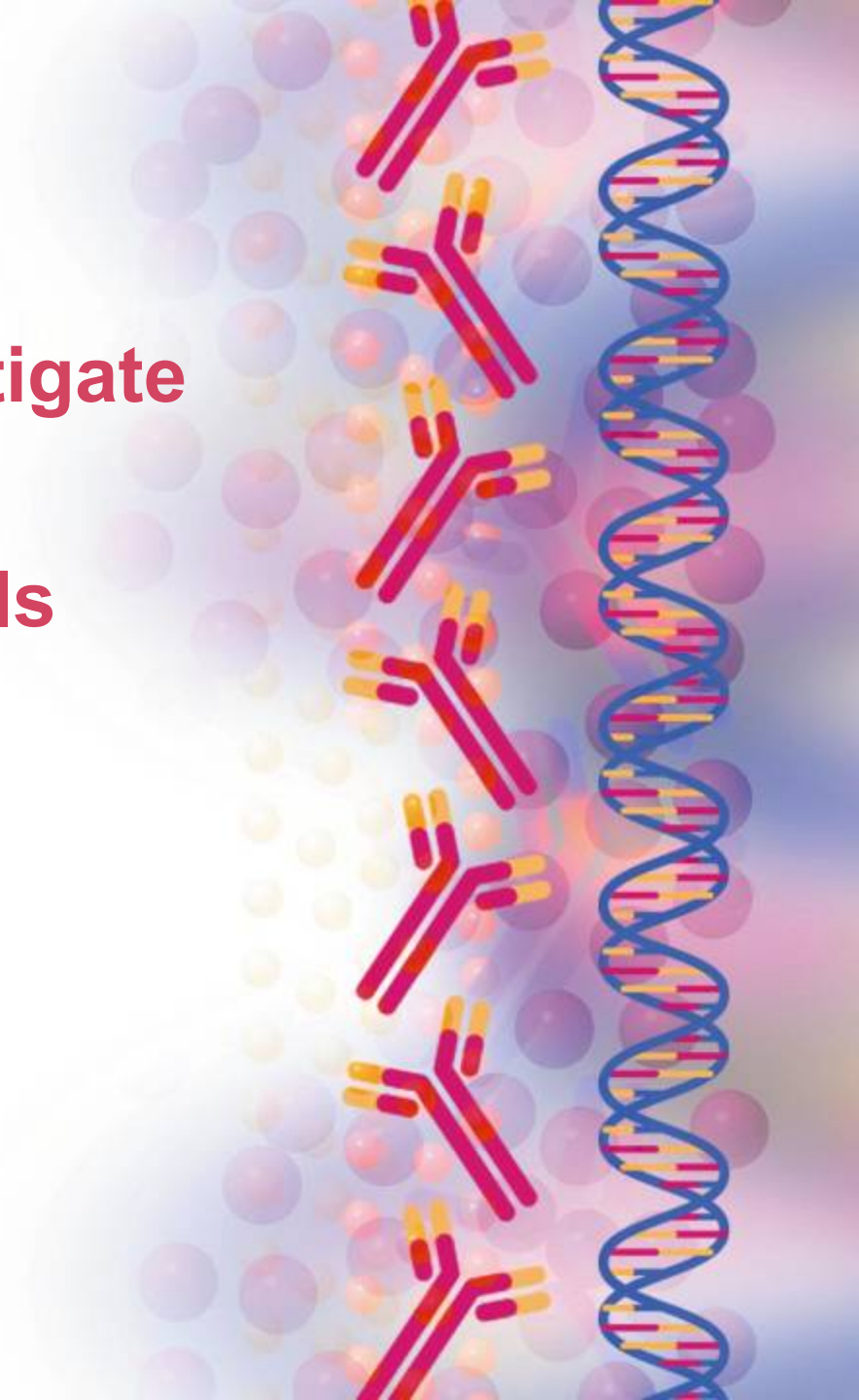
How to monitor and mitigate immunogenicity during early phase clinical trials

GEOFF HALE



Summary

- T cells orchestrate the immune response
- The established guidelines for measurement of immunogenicity are insane
- A surprisingly high proportion of healthy people have antibodies against PEG
- It may be possible to tolerise patients to prevent the formation of anti-drug antibodies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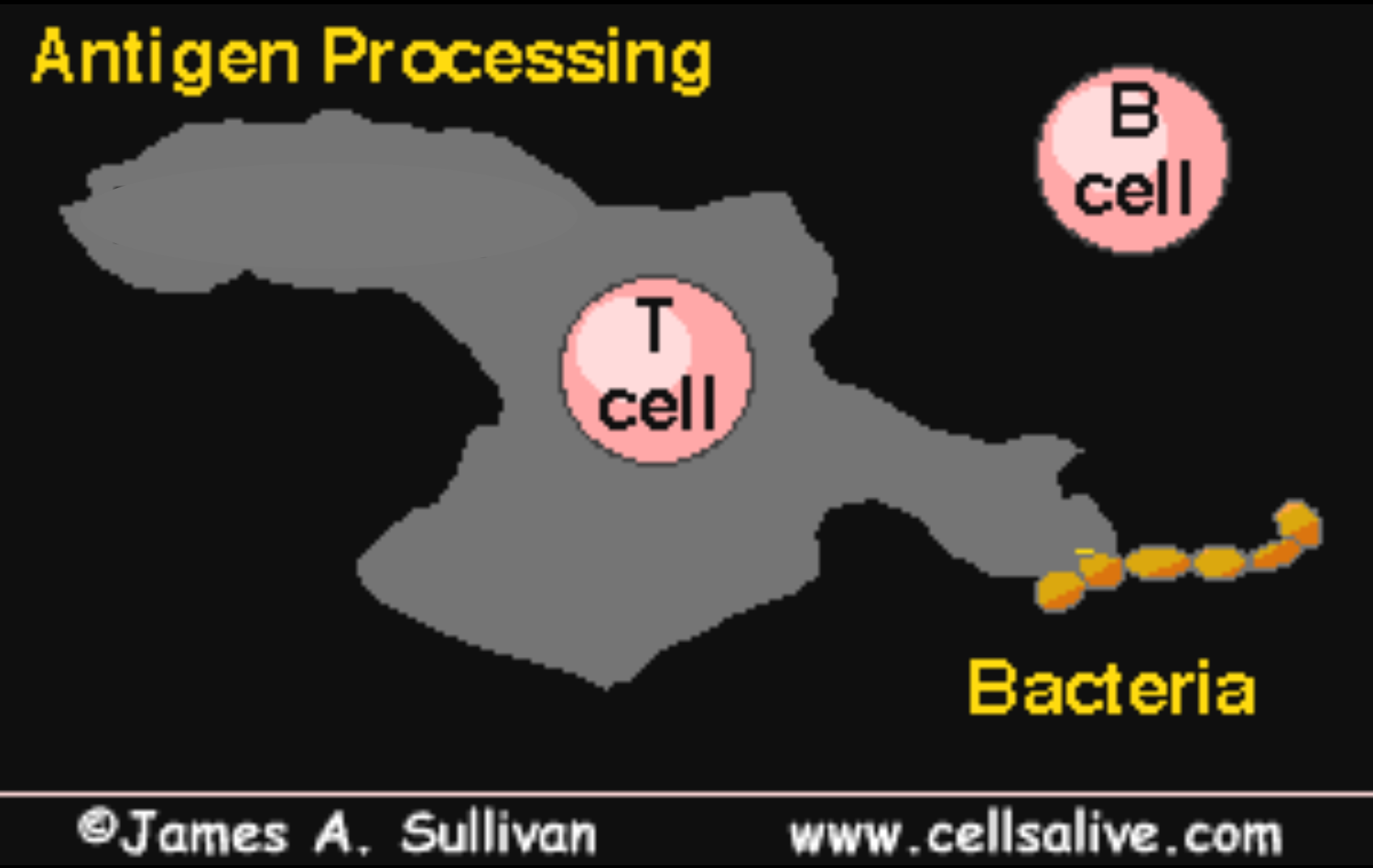
Antigen Processing
B cell
T cell
Bacteria
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PLOS ONE

RESEARCH ARTICLE

Use of *In Vitro* Assays to Assess Immunogenicity Risk of Antibody-Based Biotherapeutics

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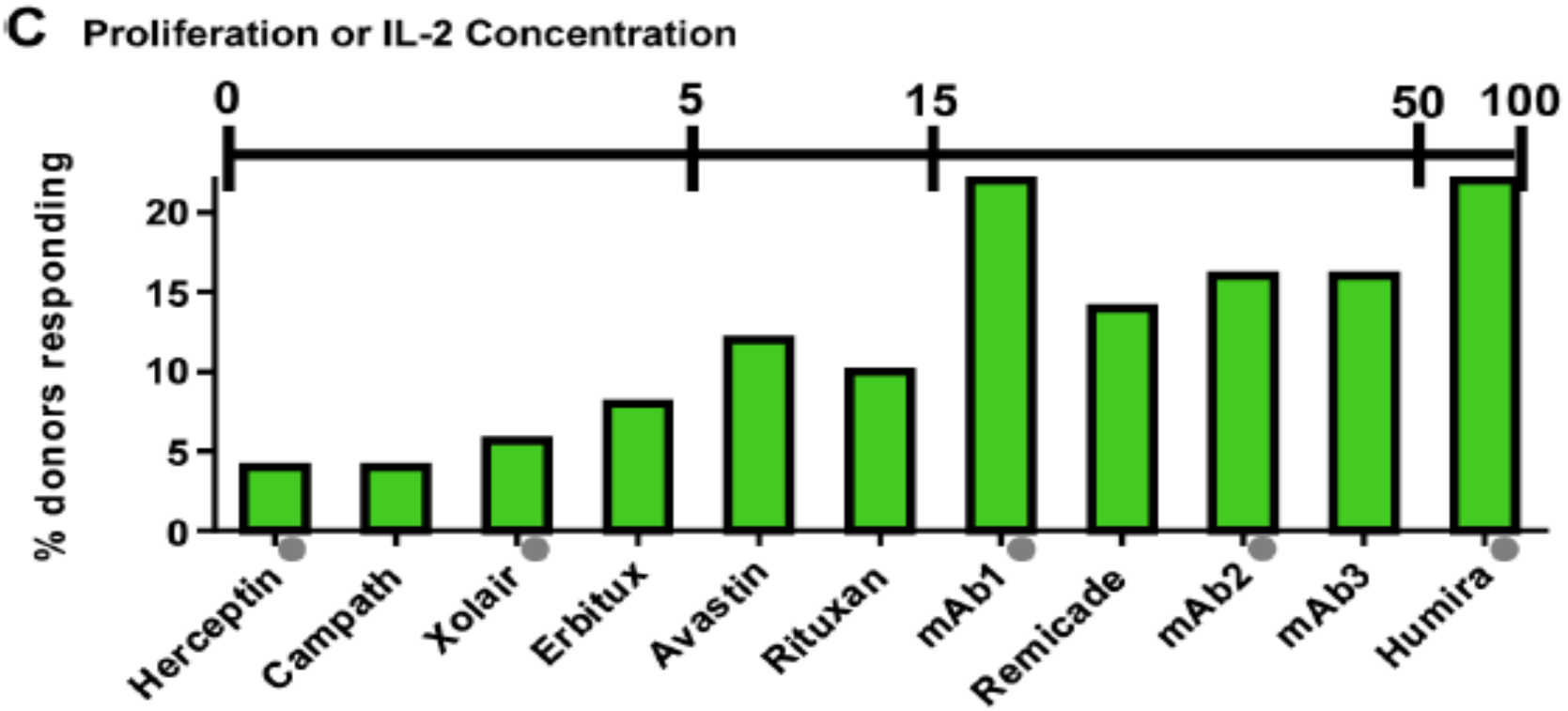
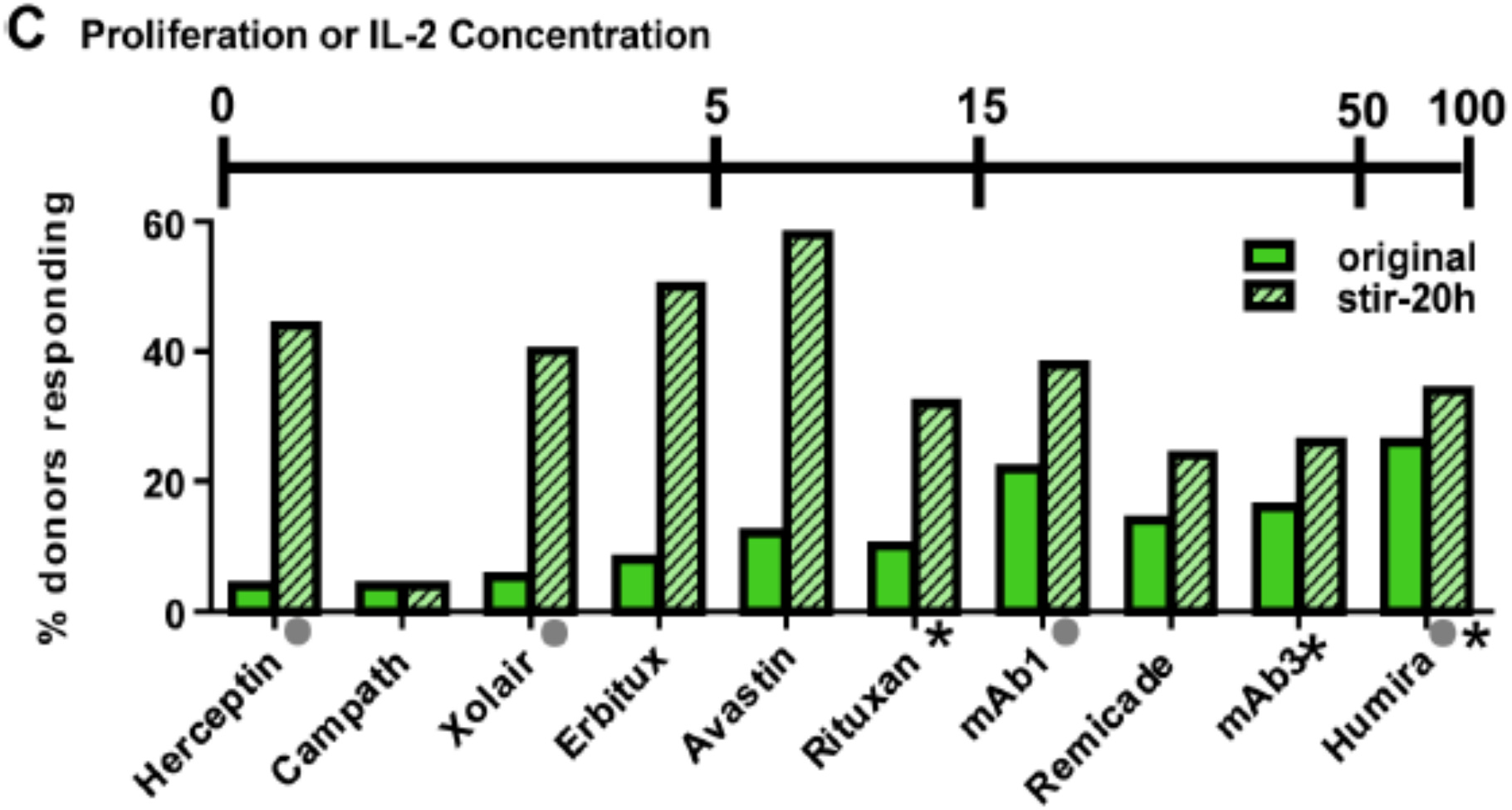
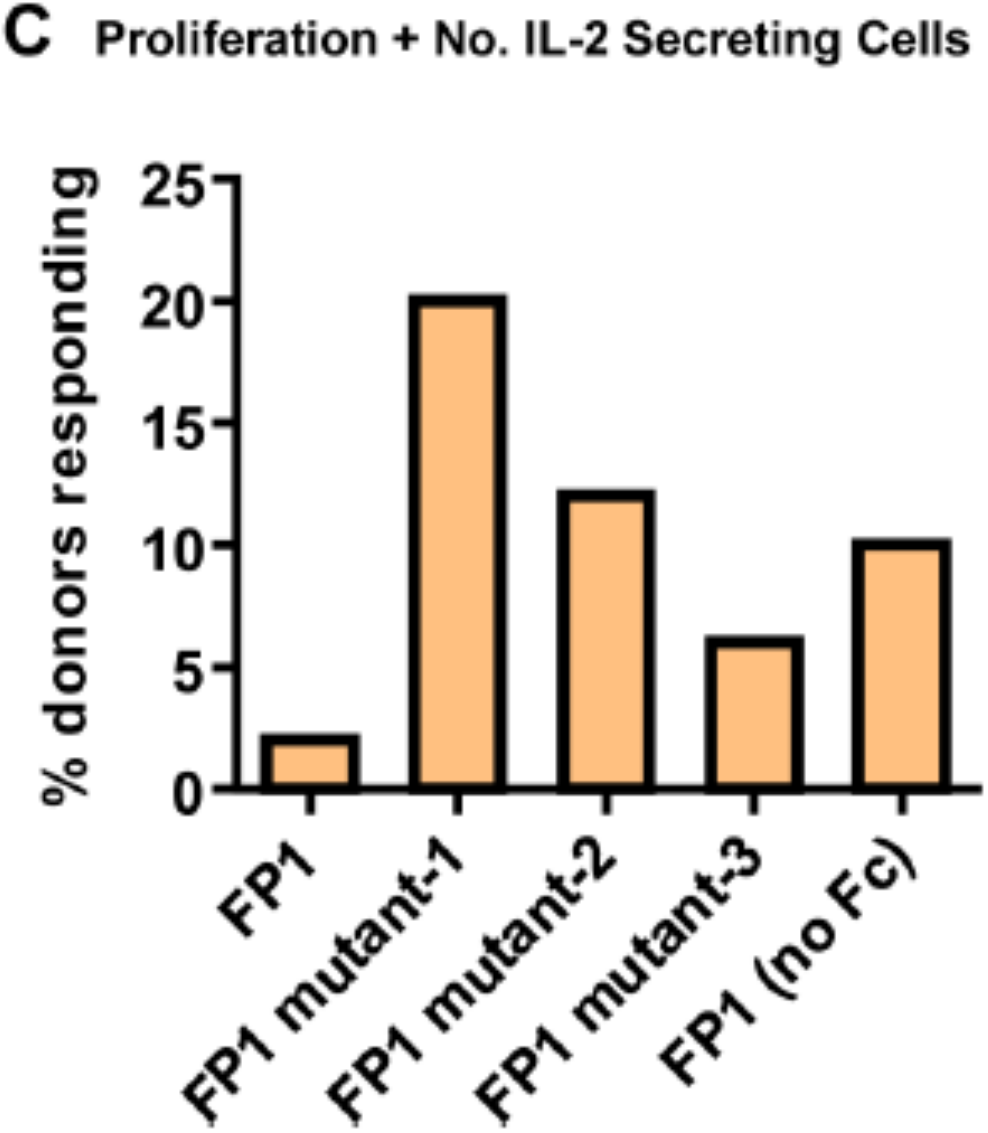
Frequency of responses correlates with frequency of anti-drug responses in the clinic



Frequency of responses increases with aggregation



Response can be sensitive to single amino acid change



Industry White Papers

Mire-Sluis AR et al. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products.

J. Immunol. Meth. 289:1-16 (2004)

Shankar et al. Recommendations for the validation of immunoassays used for the detection of host antibodies against biotechnology products.

J. Pharm Biomed Anal. 48:1267-1281 (2008)

Regulatory Guidance

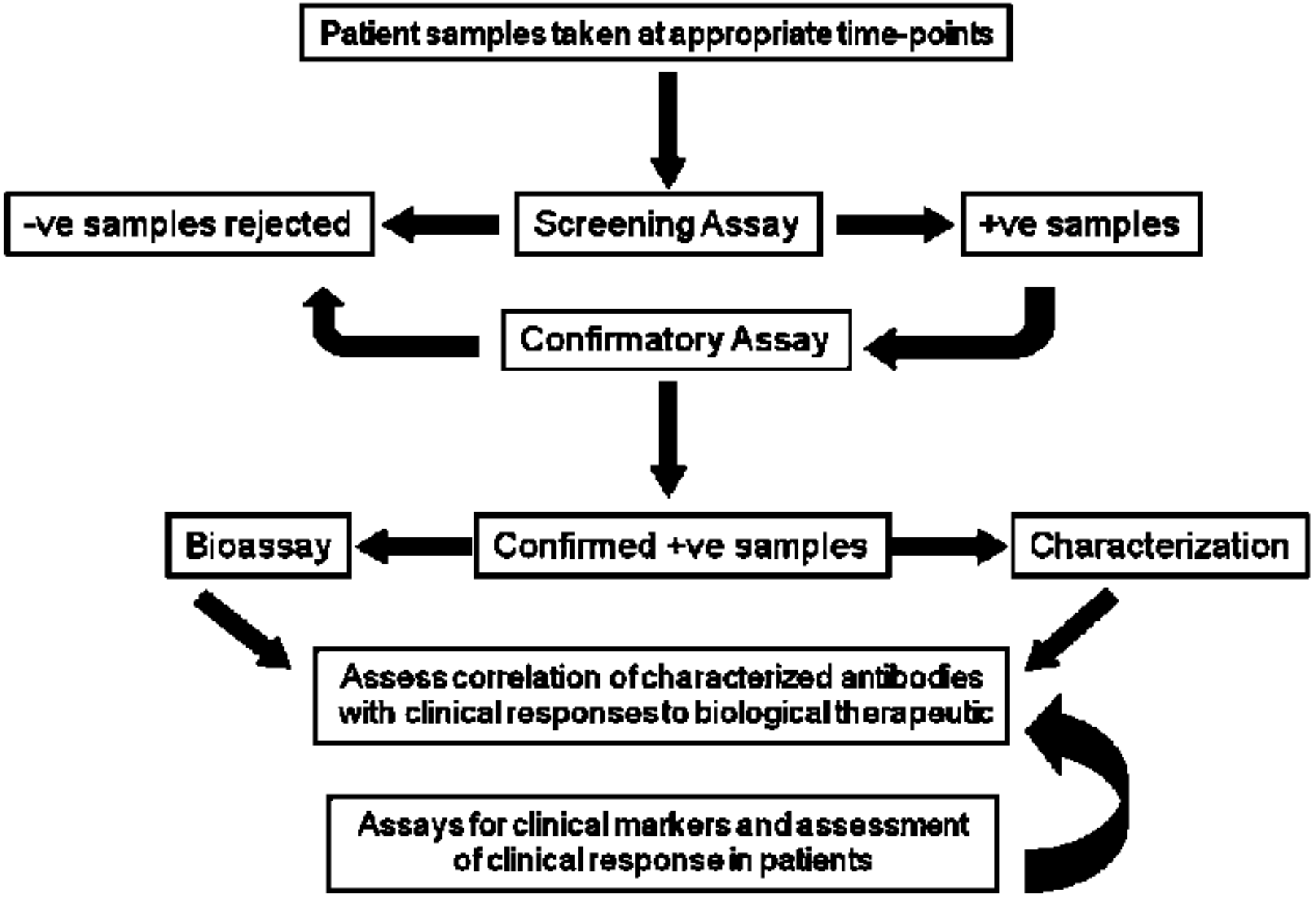
EMEA: Guideline on immunogenicity assessment of therapeutic proteins.

EMEA/CHMP/BMWP/14327/2006

EMEA: Guideline on immunogenicity assessment of monoclonal antibodies
EMA/CHMP/BMWP/86289/2010

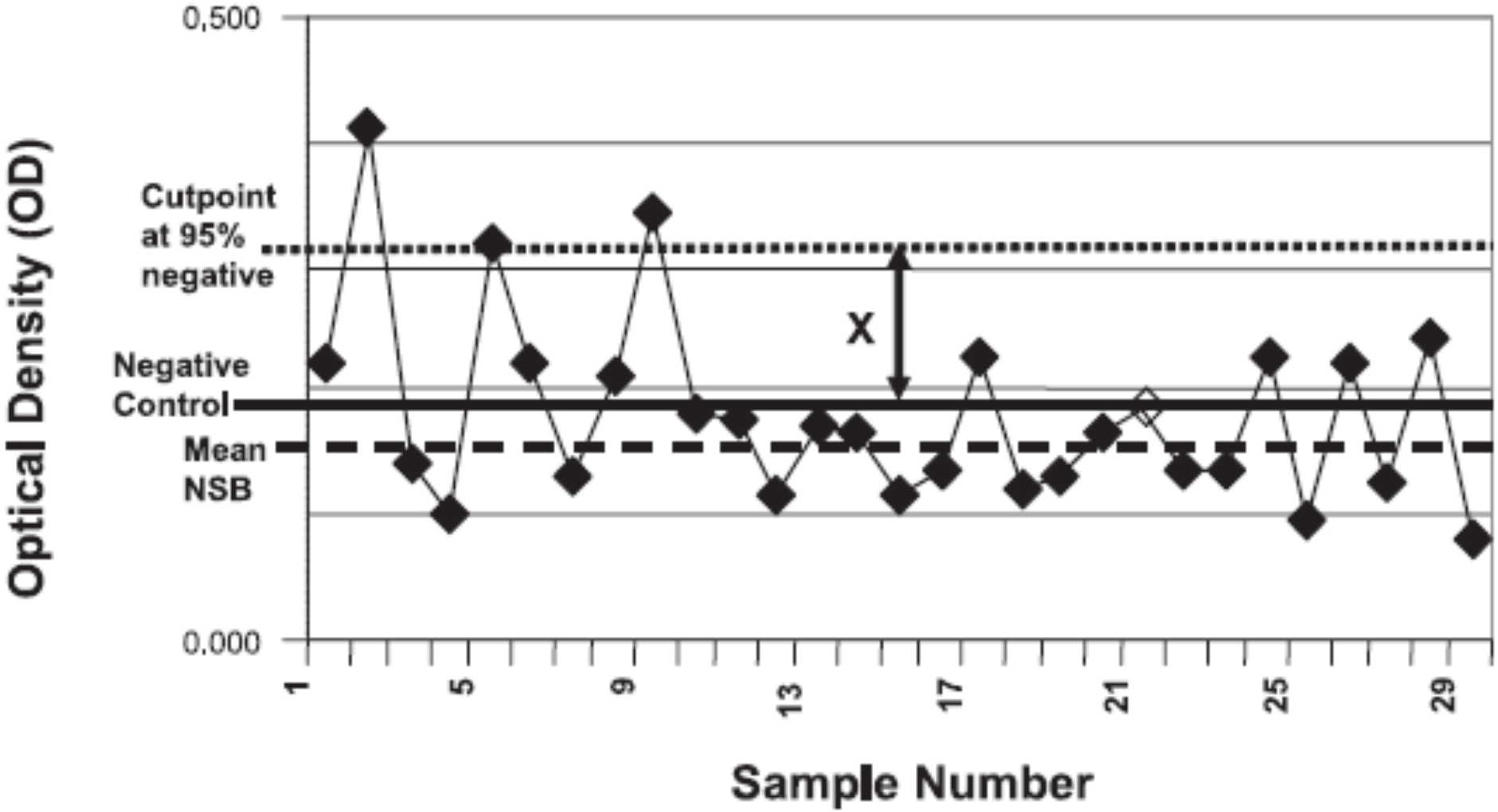
FDA: Guidance for Industry: Immunogenicity Testing of Therapeutic Proteins (2014)

FDA: Guidance for Industry: Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products (draft, 2016)


Patient samples taken at appropriate time-points
-ve samples rejected
Screening Assay
+ve samples
Confirmatory Assay
Bioassay
Confirmed +ve samples
Characterization
Assess correlation of characterized antibodies with clinical responses to biological therapeutic
Assays for clinical markers and assessment of clinical response in patients

Determination of cut-point

A.R. Mire-Sluis et al. / Journal of Immunological Methods 289 (2004) 1–16



Screening Assay: Cut point

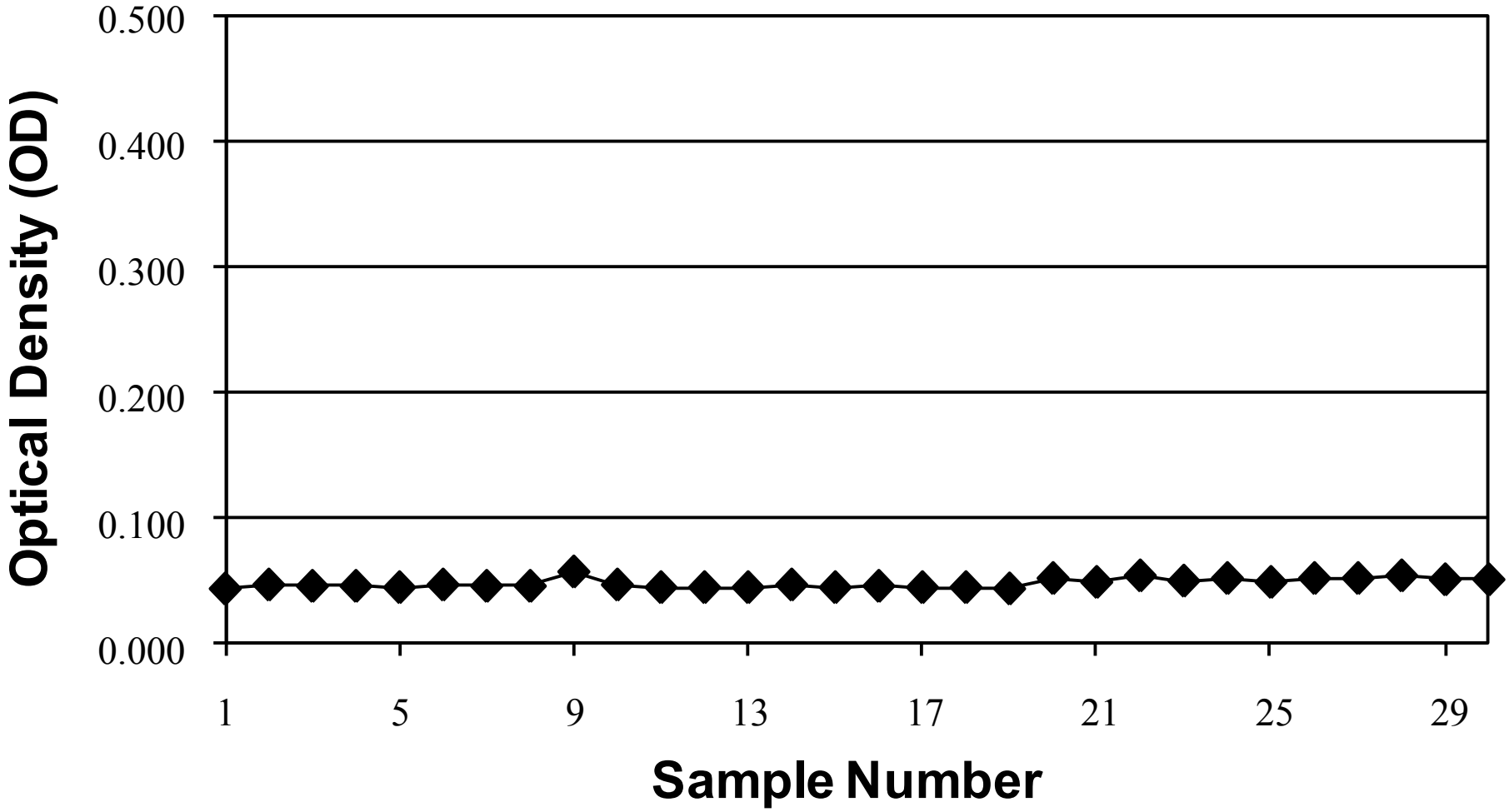
AAPS	EMEA	FDA
It is appropriate to have 5% false positives	Detection of some false positive results is inevitable	recommends … a 5% false positive rate. The approach … will depend on various factors

Screening Assay: Sensitivity

AAPS	EMEA	FDA
Strive for sensitivities near 250 to 500 ng/mL	Capable of detecting antibodies in all antibody-positive samples/patients	Traditionally recommended at least 250 to 500 ng/mL Now recommends at least 100 ng/mL

Determination of cut-point

Data from Ingrid Caras, PDL



Case Study 1: What Happened

- Ran post-dose Study samples
- Applied cut point (OD ~0.06)
- High incidence of 'positives' with low OD values
  - a few hundredths of OD unit above cut point
- Most were sporadic or 'transient'
  - Pos at one time point and Neg at the next
- Not typical of a real immune response
- Impossible to confirm in a competition assay
  - OD's too close to the floor

Subject Example

Day	OD	
0	0.067	
14	0.087	*Pos*
28	0.074	
56	0.076	*Pos*
84	0.066	

Case Study 1: Conclusion

- Strict adherence to the statistical approach rules would mean reporting these subjects as *positive* – resulting in high incidence
- BUT
  - *Most positives based on few hundredths of OD unit above cut point*
  - *Sporadic timing didn't look like a real immune response*
  - *Previous studies showed very low immunogenicity*
- Important to apply some common sense
- Combination of tight assay with v low background and v low SD resulted in cut point that was too low
- Result – we pick up a lot of 'noise'

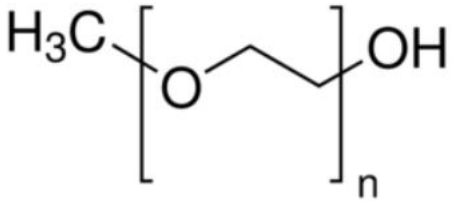
Subject Example

Day	OD	
0	0.067	
14	0.087	***Pos***
28	0.074	
56	0.076	***Pos***
84	0.066	

Cut Point and Sensitivity

Insane	Sane
5% false positive	Minimise false positive and false negative.
Arbitrary sensitivity based on assay variability	Optimal sensitivity (100 ng/mL) based on likelihood of clinical sequalae
Impossible to compare different assays	Assay results can be compared
Assay development and validation is exceedingly cumbersome	Assay development and validation is simple
Any sort of in house reference might be used	Reference should be optimised and well characterised

Polyethylene glycol



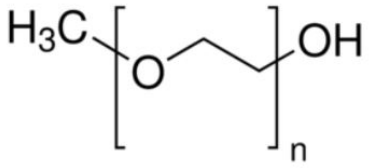
(aka polyethylene oxide, polyoxyethylene)

Phamaceutical: laxative, eye drops, excipient, protein modifier

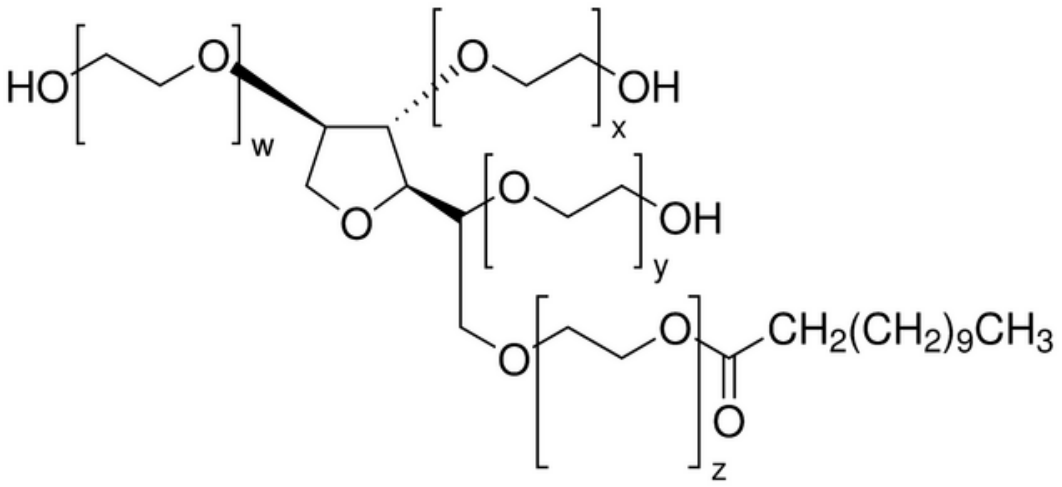
Commercial: wood preservatives, paints, rocket fuel,
gas scrubber in power plants, anti-foaming agent,
ceramic manufacture

Domestic: tooth paste, skin creams, lubricants, inkjet printers,
paintballs, anti-foaming agent in food, e-cigarettes

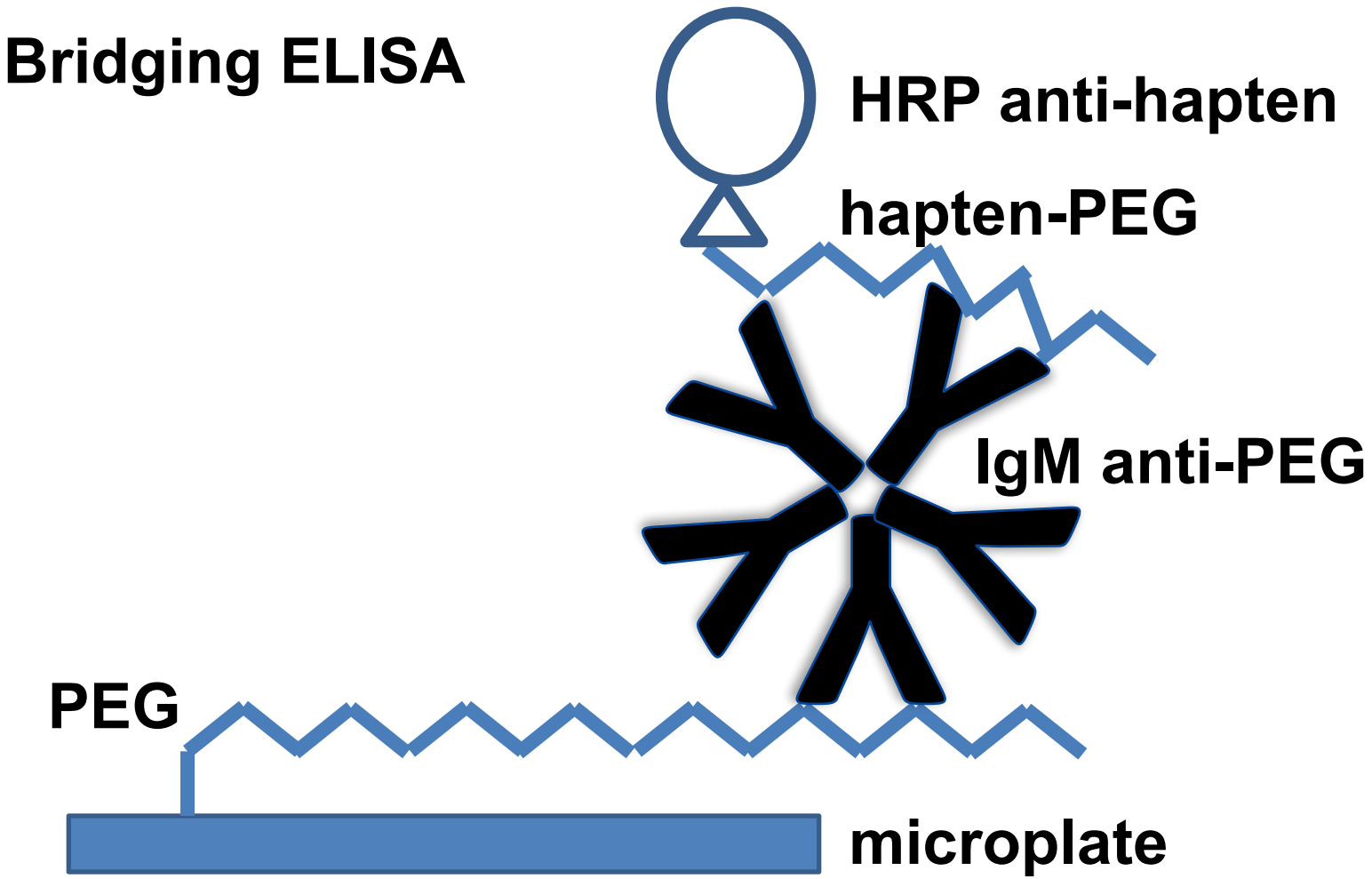
Detergents containing polyethoxy groups



Tween 20

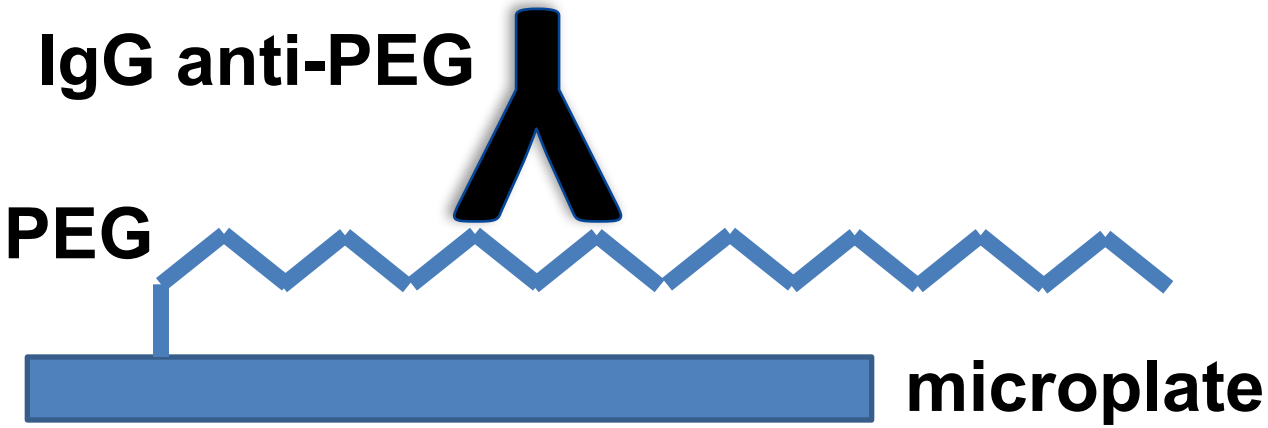


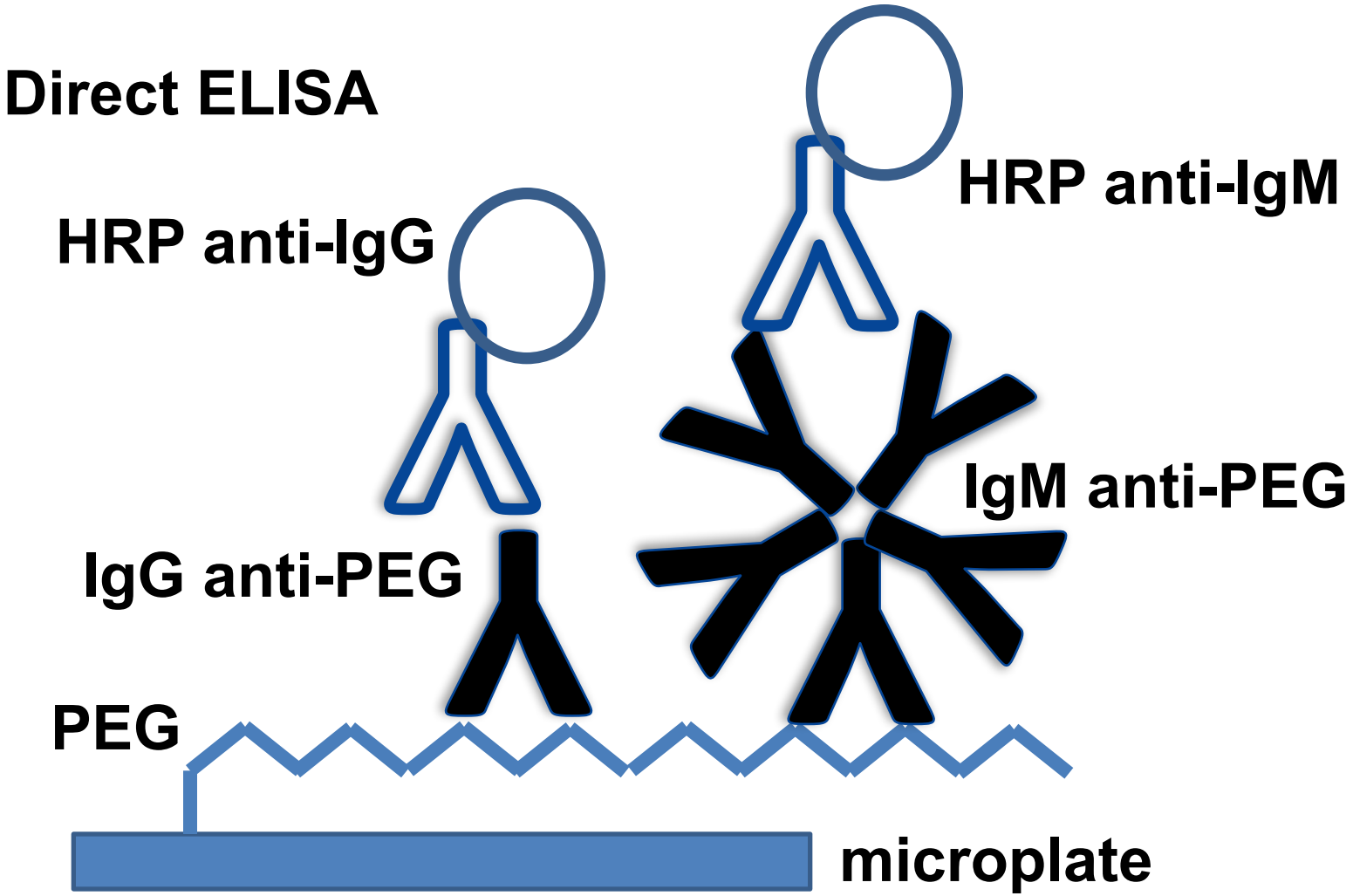
also:
Nonidet, Pluronic, Polysorbate, Triton


Bridging ELISA
HRP anti-hapten
hapten-PEG
IgM anti-PEG
PEG
microplate

Bridging ELISA

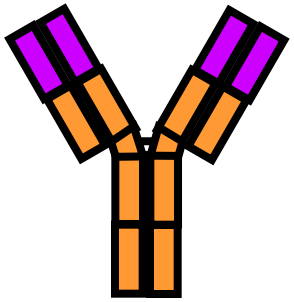
Detection not possible!



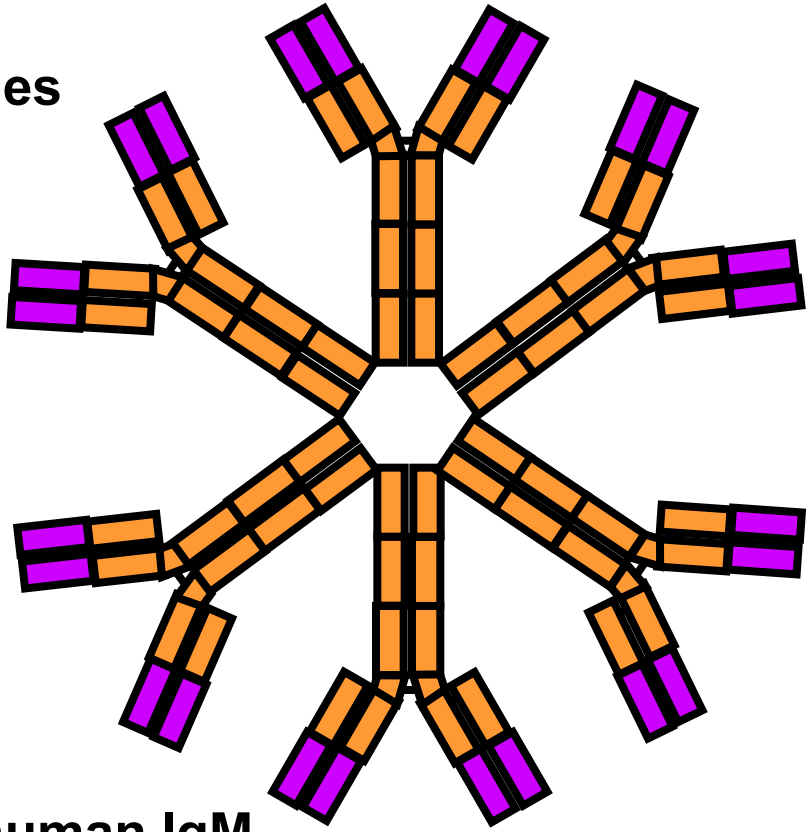

Direct ELISA
HRP anti-IgM
HRP anti-IgG
IgM anti-PEG
IgG anti-PEG
PEG
microplate

absolute antibody

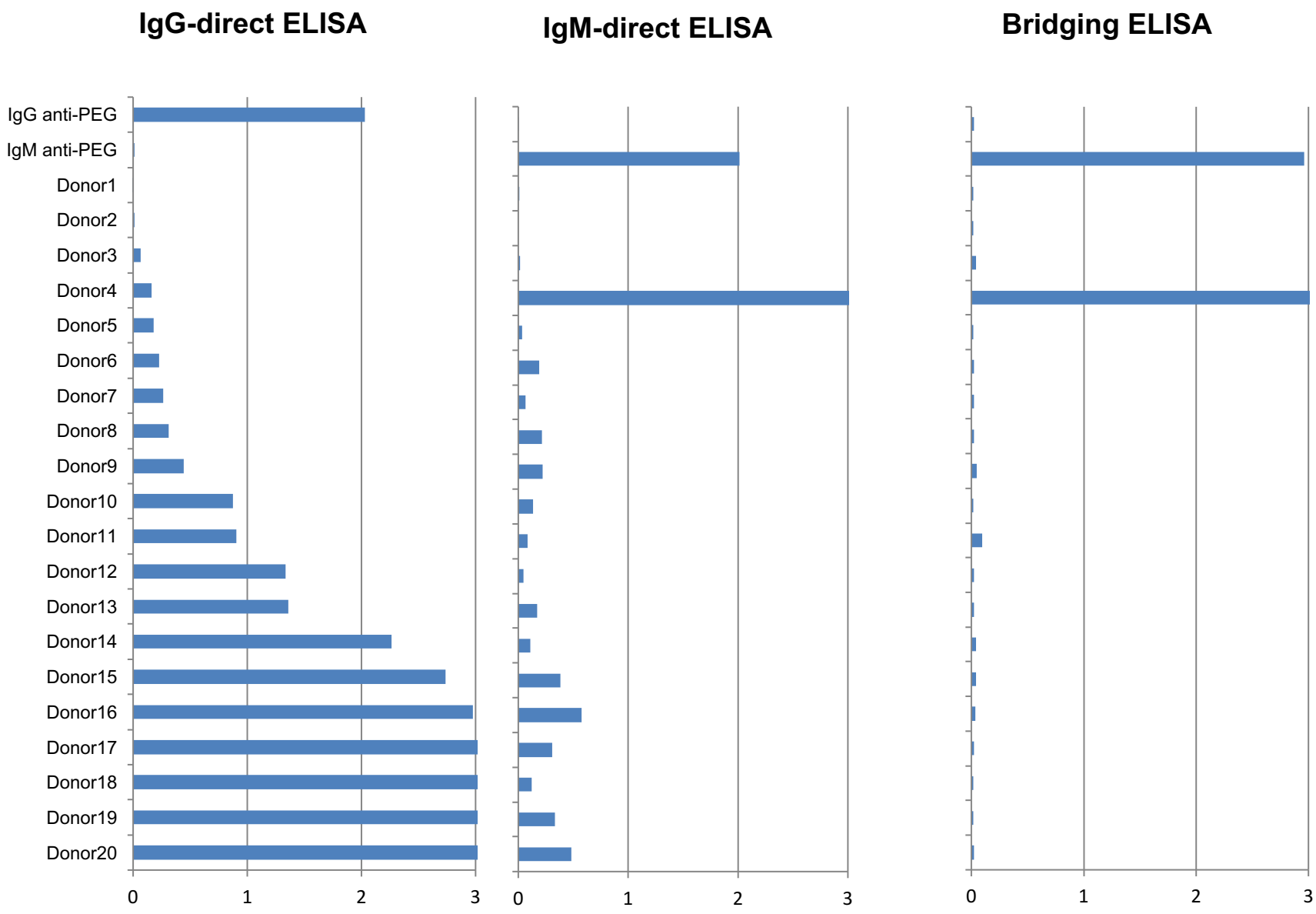
Anti-PEG control antibodies



chimeric human IgG1



chimeric human IgM


IgG-direct ELISA
IgM-direct ELISA
Bridging ELISA
IgG anti-PEG
IgM anti-PEG
Donor1
Donor2
Donor3
Donor4
Donor5
Donor6
Donor7
Donor8
Donor9
Donor10
Donor11
Donor12
Donor13
Donor14
Donor15
Donor16
Donor17
Donor18
Donor19
Donor20
0
1
2
3

Proportion and titre of anti-PEG antibodies in 50 healthy individuals

Titre	Number (%)	
	IgG	IgM
Negative	9 (18%)	20 (40%)
Positive	41 (82%)	30 (60%)
10-20	21	20
40	0	2
80	0	2
160	3	2
320	3	0
640	6	1
1280 or greater	8	3

Effect of Tween in wash buffer

	Absorbance			
Sample	IgG anti-PEG		IgM anti-PEG	
	PBS	PBS-Tween	PBS	PBS-Tween
M-A05	3.187	0.020	2.886	3.149
M-A07	3.015	-0.049	0.151	0.379
M-A09	2.967	0.004	0.439	0.598
F-A03	3.095	0.062	-0.194	0.175
F-A05	3.008	-0.035	-0.013	0.076
IgG control	1.142	0.502		
IgM control			1.484	1.987

Conclusions

- Unexpectedly high frequency and titre of IgG anti-PEG antibodies in healthy donors
- Previous assays would not have detected them
- Consequence of exposure to PEG in the environment?
- Implications for PEG in drug conjugates?
- Utility of recombinant control reagents
- Beware of using bridging assay if the antigen has repeating epitopes
- Traditional cut-point approach cannot be used when there are pre-existing antibodies

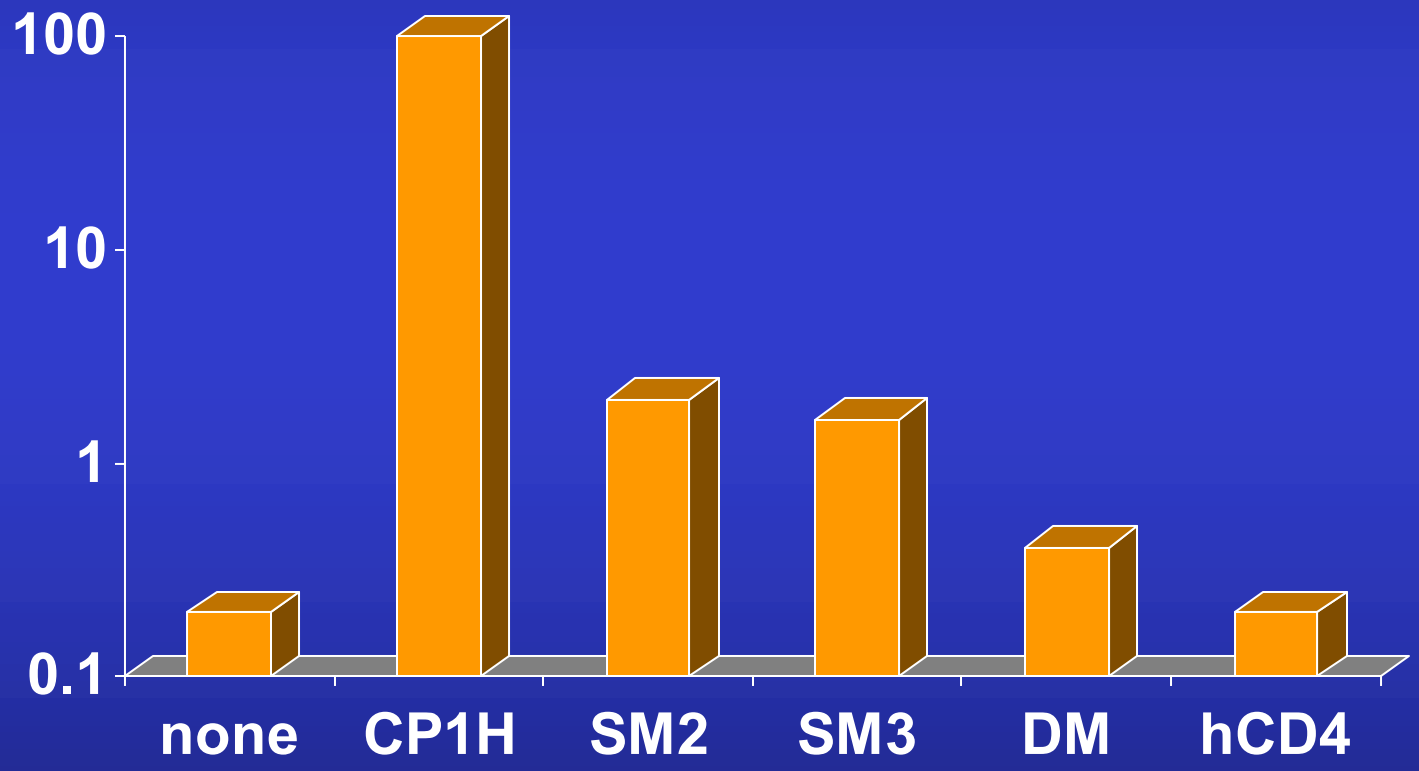
Prevention of unwanted antibodies by induction of tolerance

- High-zone tolerance to deaggregated IgG
- High response to cell-binding antibodies
- Tolerance induced by non-binding antibodies

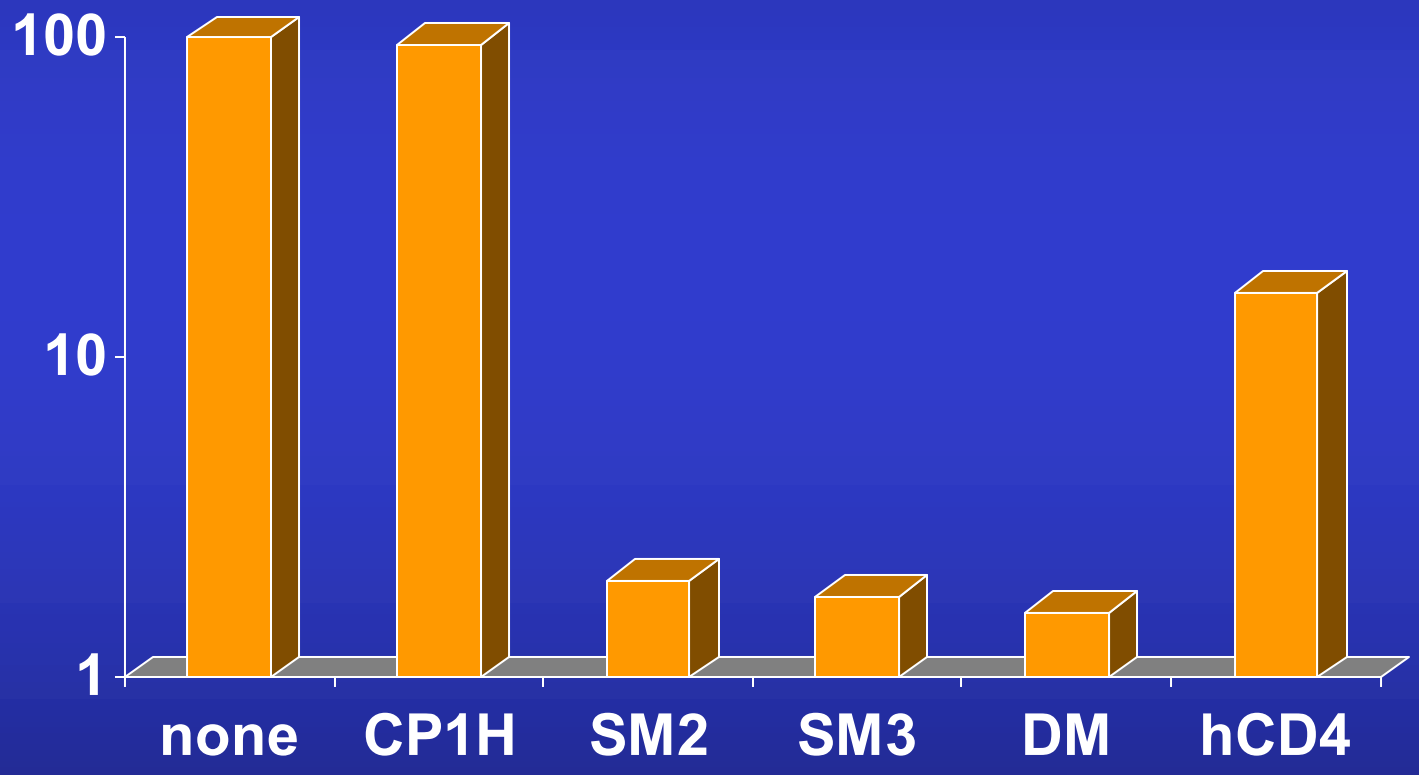
alemtuzumab mutants

	V_H CDR2 sequence	Binding
Wild-type	R D K A K G	100 %
SM1	* * D * * *	50%
SM2	* K * * * *	10%
SM3	* * * * D *	< 1%
DM	* * D * D *	< 1%

Immunogenicity of mutants in vivo



Responses to wild-type alemtuzumab



Clinical Trial of SM3 to induce tolerance

- Proof of concept study in 15 patients
- High dose SM3 followed by standard course of treatment with alemtuzumab (5 days)
- Second cycle of treatment after 12 months