Pre-existing antibodies to biologics: Predictive for treatment outcome?



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Appearance of serum sickness-like reactions during treatment with rituximab

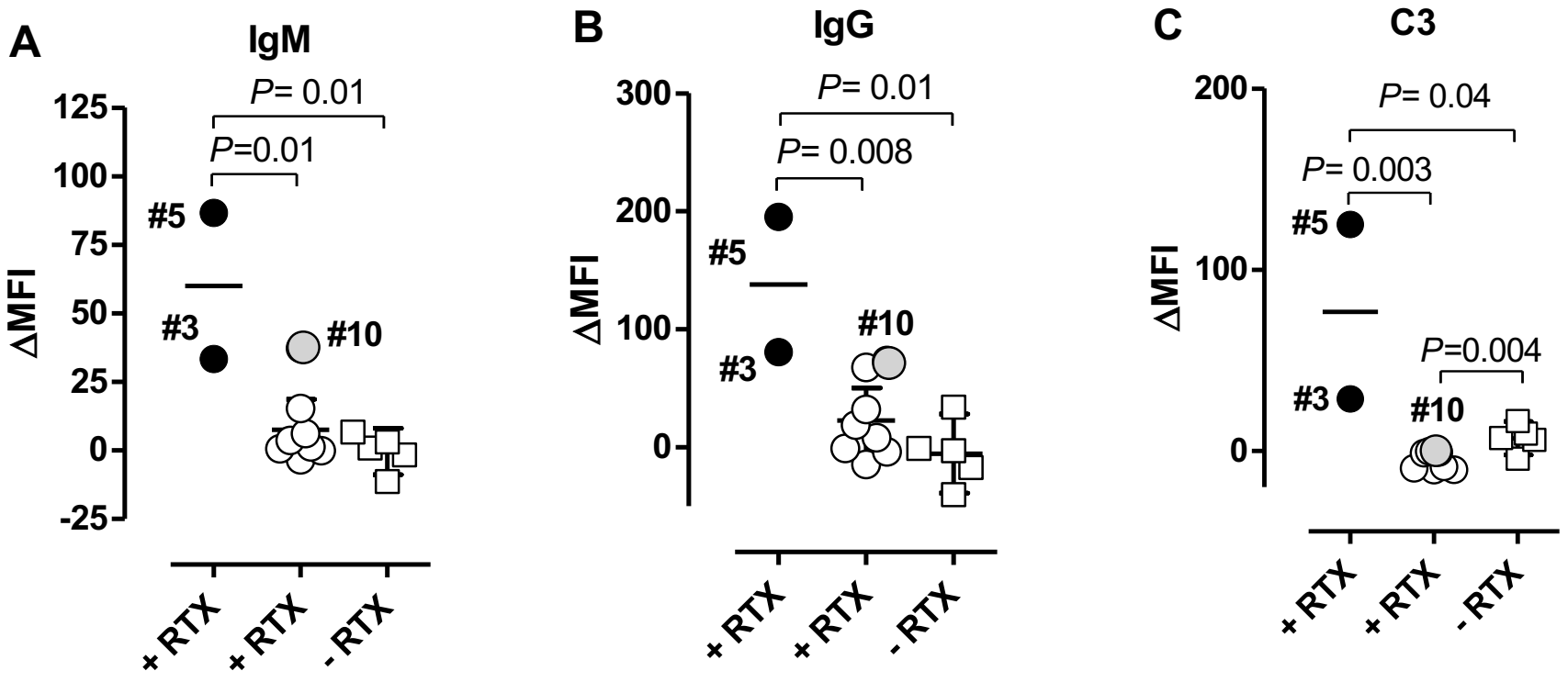
- In a study on Sjögrens disease HACAs were formed in 4 of 8 patients, 3 of these developed SSLR (Pijpe J, Arthritis Rheum 2005)
- In children with ITP, RTX caused SSLRs in 5 out of 60 (Bennet et al. Blood 2006; Wang et al., J Pediatr 2005).
- In a study on Graves' disease 3 of 10 patients developed SSLR (el Fassi et al.,J Endocrin Invest, 2011)

Serum sickness like reactions (SSLR)



Arthralgia, skin eruptions, fever, lymphadenopathy

Demonstration of circulating complement-opsonized immune complexes



An anti-C3-capture ELISA developed with anti-IgG/IgA/IgM confirmed

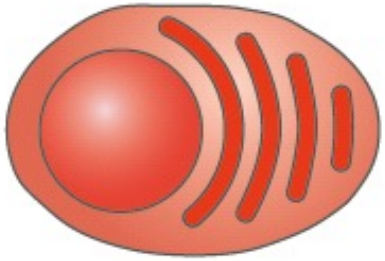
El Fassi et al. J Endocrinol Invest 2011; 34: e163-e167

Basic conundrum

How can you develop antibody responses
when no B cells are present?

Is there a pre-existing immune response to rituximab?

Ongoing production of pre-existing antibodies by plasma cells?



Study on endometrial protein PP14:

81% of male sera were false-positive

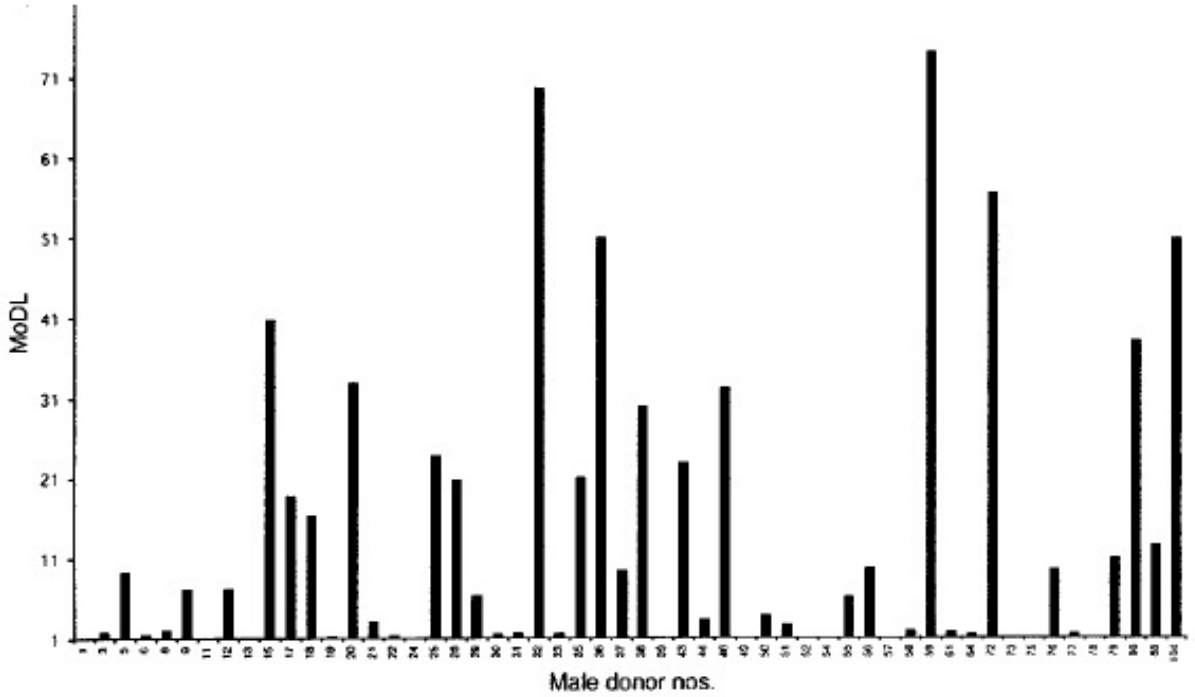
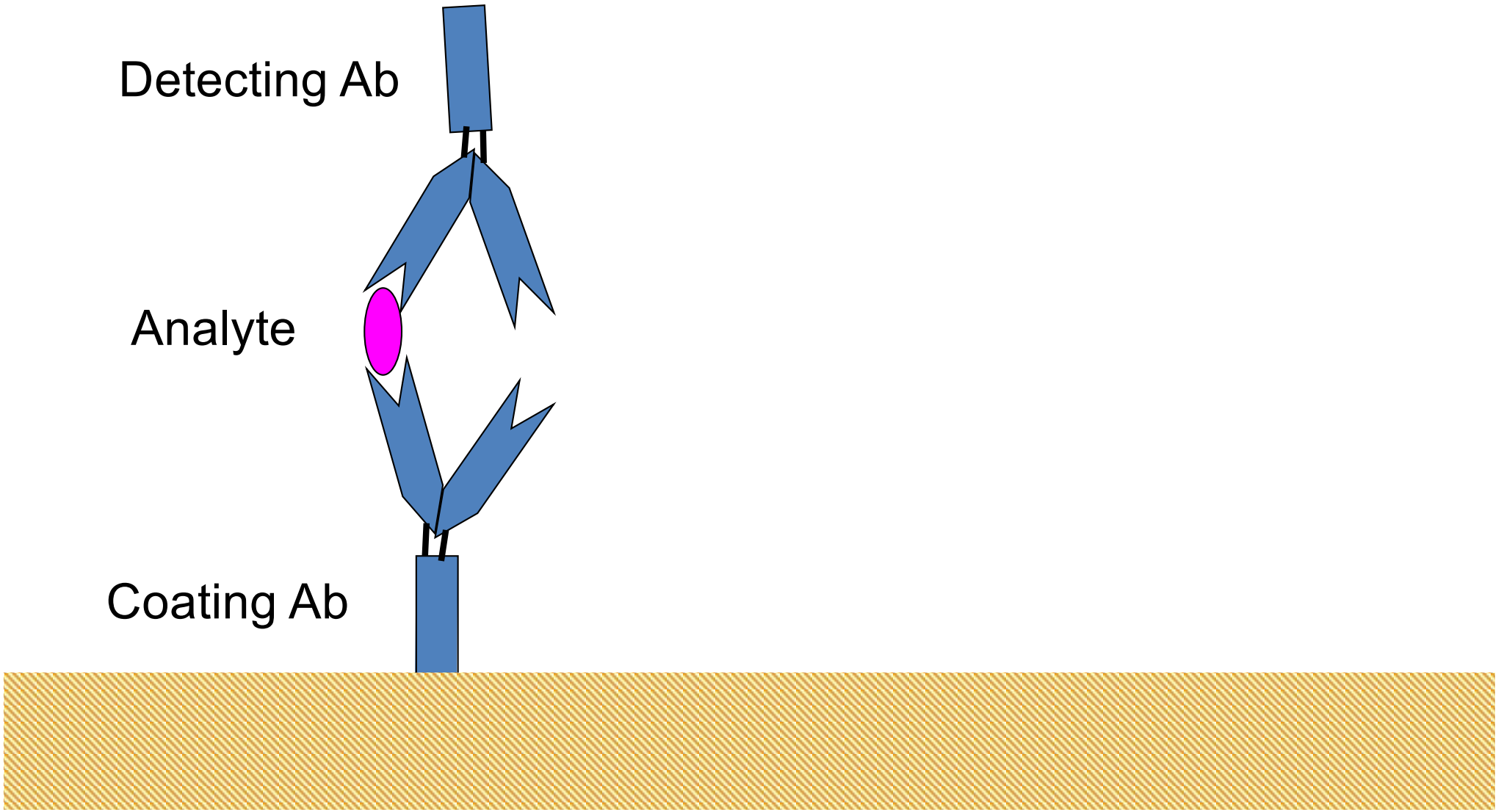


Figure 1. The signals obtained when 54 male sera were analyzed in the PP14 ELISA based on Mab. The signals are expressed as MoDL, the detection limit being defined as the mean ($n = 20$) of serum blank + 3SD.

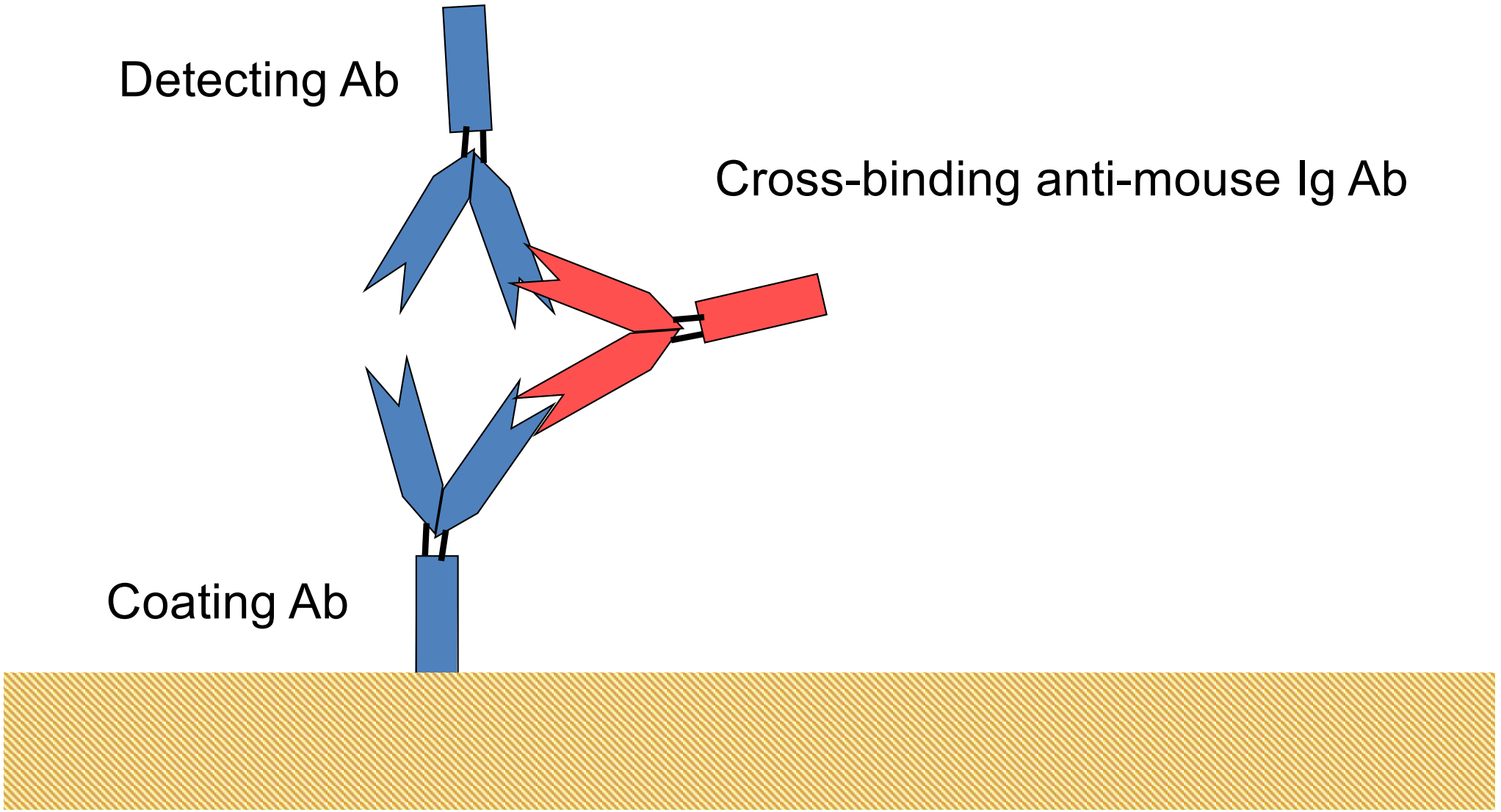
Ditte C. Andersen, Claus Koch, Charlotte H. Jensen, Karsten Skjødt, Jette Brandt, and Børge Teisner*

JOURNAL OF IMMUNOASSAY & IMMUNOCHEMISTRY
Vol. 25, No. 1, pp. 17–30, 2004

False-positive reactions in two-site immunoassays



False-positive reactions in two-site immunoassays



Kricka et al. Clin Chem 1999; 45: 942-56

Prevalence of antibodies causing false-positive reactions

Prevalence	Population	Ref.
Human anti-mouse IgG		
76%	67 blood donors	*87*
80%	10 infants	*87*
0.72%	10 000 blood donors	*88*
9.12%	1008 blood donors	*89*
Human anti-rabbit IgG		
5%		*90*
0.13%	10 261 neonates	*38*
0.09%	75 734 neonates	*89*
0.3%	9241 infants	*37*
Human anti-chicken IgG		
0%	150 patients and blood donors	*91*
Human anti-bovine albumin		
43%	28 healthy subjects	*92*
Human anti-bovine IgG		
7%	Blood donors	*93*
Human anti-sheep IgG		
7%	Blood donors	*93*
Human anti-guinea pig IgG		
0.51%	Blood donors	*4*
Multivalent antibody-binding substances[a]		
40%	668 blood donors, laboratory personnel, and patients	*5*

[a] Antibody-binding substances are substances that react with a capture and a labeled detection antibody to form a sandwich.

What are they?

HAAAs: Human anti-animal antibodies

HAMAs: Human anti-mouse antibodies

HACAs: Human anti-chimeric antibodies

HAHAs: Human anti-human antibodies

Heterophilic antibodies

HAAAs versus Heterophilic Antibodies

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Stanley S. Levinson[1,2*]

[1] *Department of Pathology and Laboratory Medicine University of Louisville Louisville, KY 40292*

[2] *Laboratory Service Veterans Affairs Medical Center 800 Zorn Ave. Louisville, KY 40206-1466*

1) Antibodies should be called heterophile when there is no history of medicinal treatment with animal immunoglobulins or other well-defined immunogens and the interfering antibodies can be shown to be multispecific

2) Antibodies should be called specific HAAAs when there is a history of treatment with animal immunoglobulin and immunoglobulin from the same species is used in the test.

Clinical Chemistry 45, No. 5, 1999

Heterophilic antibodies

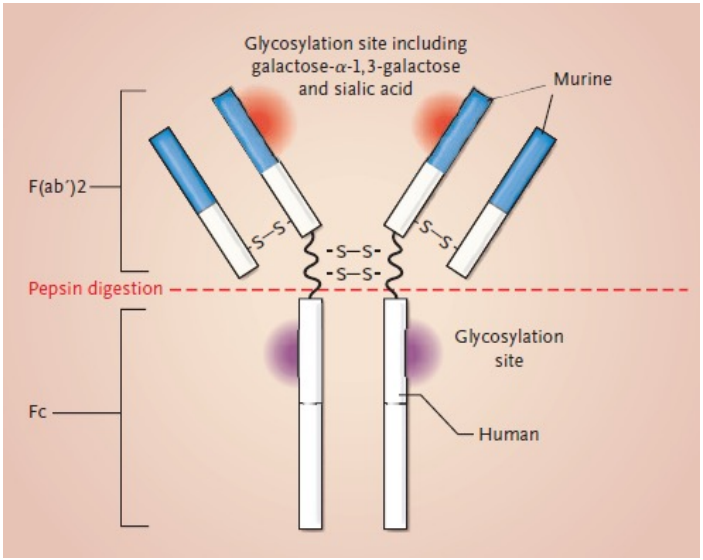
Immunoglobulin	Group I Anti-Fab (85% of positive samples)	Group II Anti-Fc (15% of positive samples)	Negative samples
Mouse	+++	+++	-
Rat	+++	-	-
Goat	+++	-	-
Horse	+++	+++	-
Rabbit	-	+++	-
Cow	+++	+++	-
BSA (neg. Control)	-	-	-

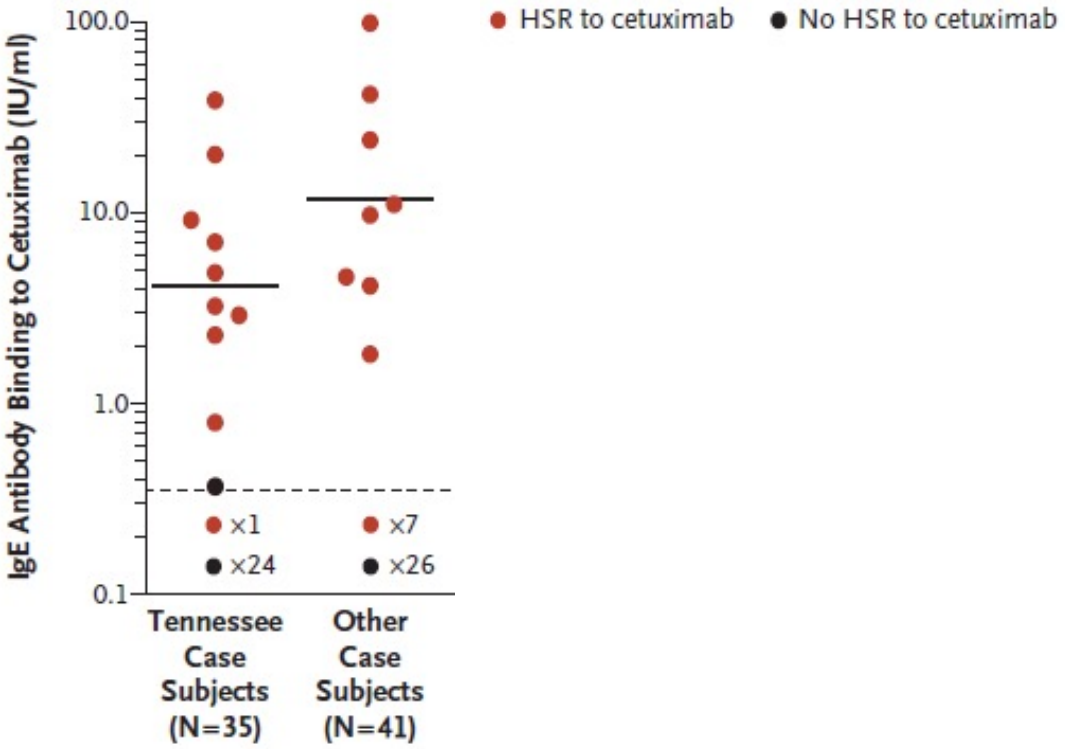
Hennig et al. Journal of Immunological Methods 235 (2000) 71–80

Chung et al., N Engl J Med 2008; 358;1109-17

Anaphylactic reactions to cetuximab caused by pre-existing IgE antibodies to the α-gal epitope

Produced in mouse hybridoma cells





All humans have IgG antibodies specific for the oligosaccharide galactose-α-1,3-galactose, which is closely related to substances in the ABO blood group

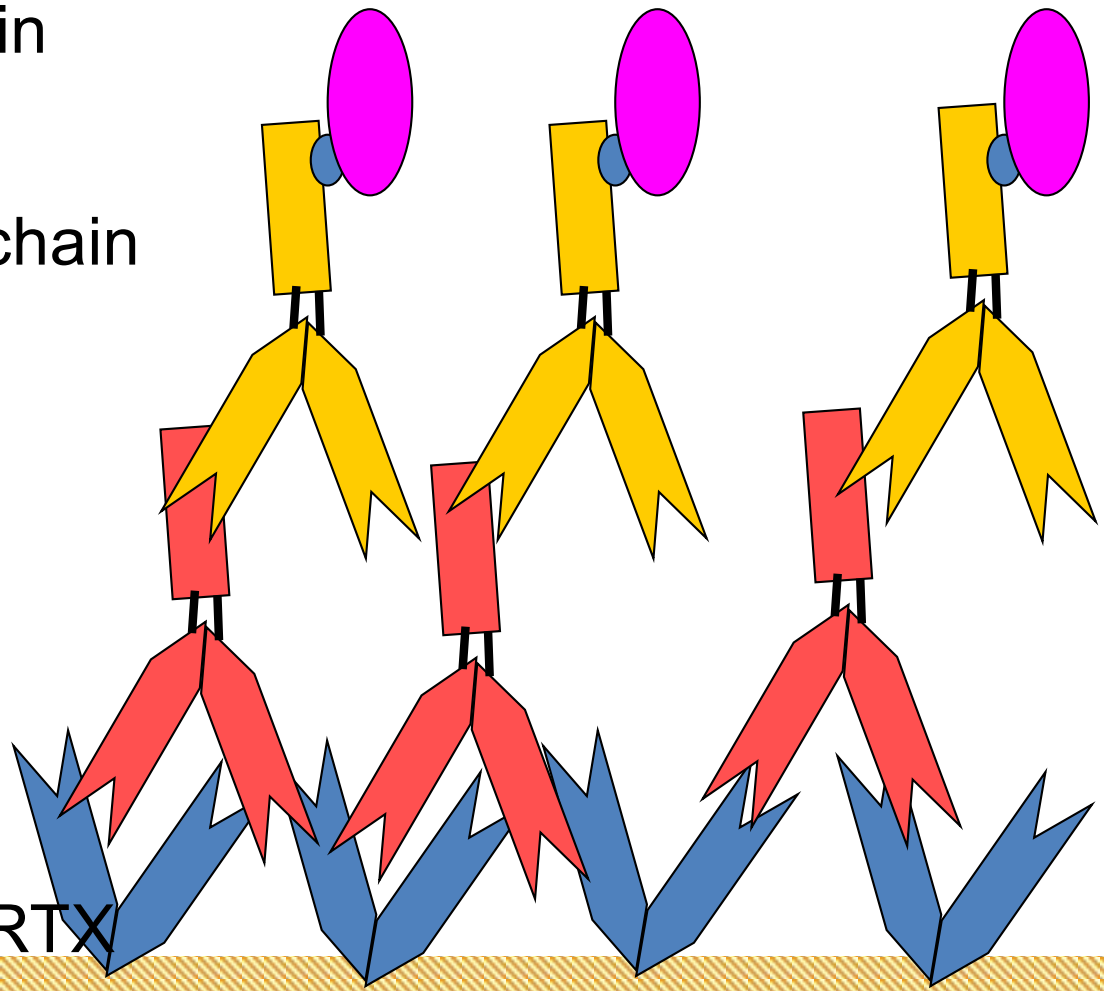
Our assay

HRP-straptavidin

Anti-human γ-chain biotin

Proband serum/plasma

Coating: $F(Ab')_2$ of IFX, RTX or murine IgG



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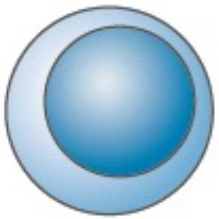
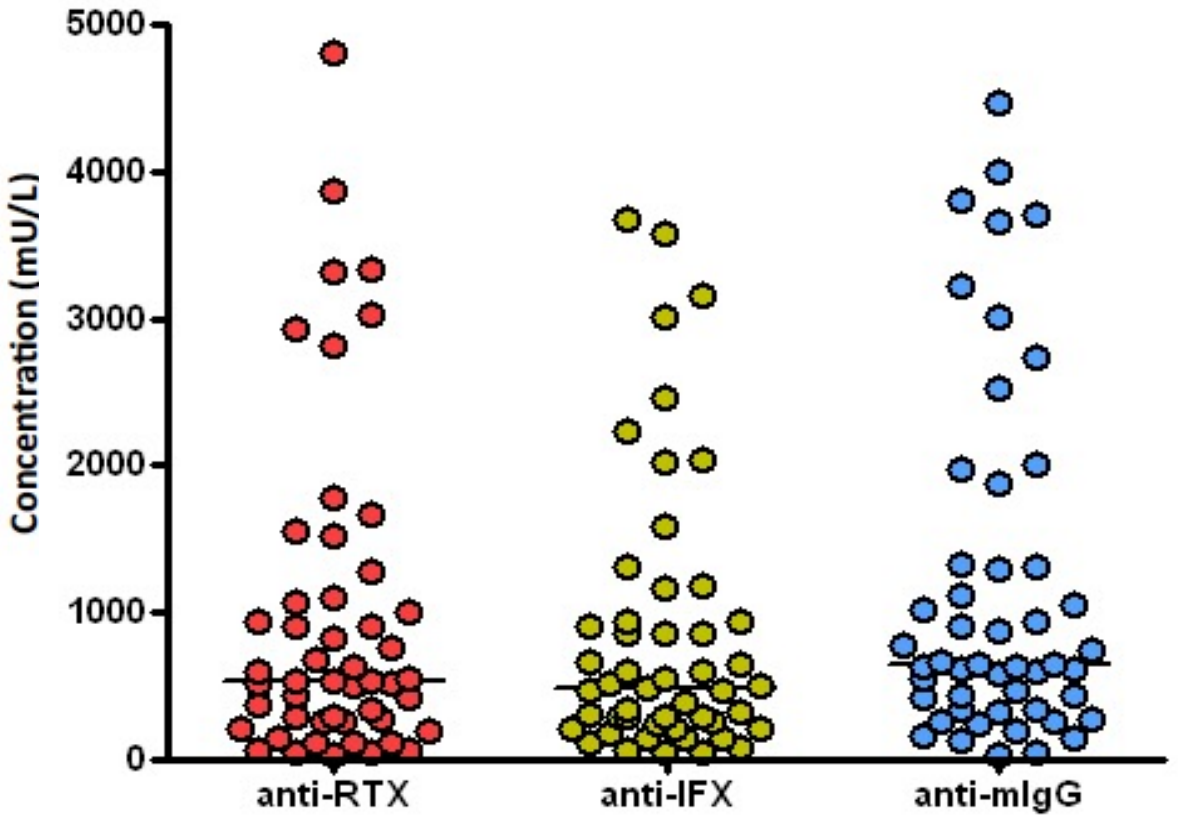
IgG antibodies against mouse Fab, anti-IFX Fab and anti–RTX Fab are common in human plasma

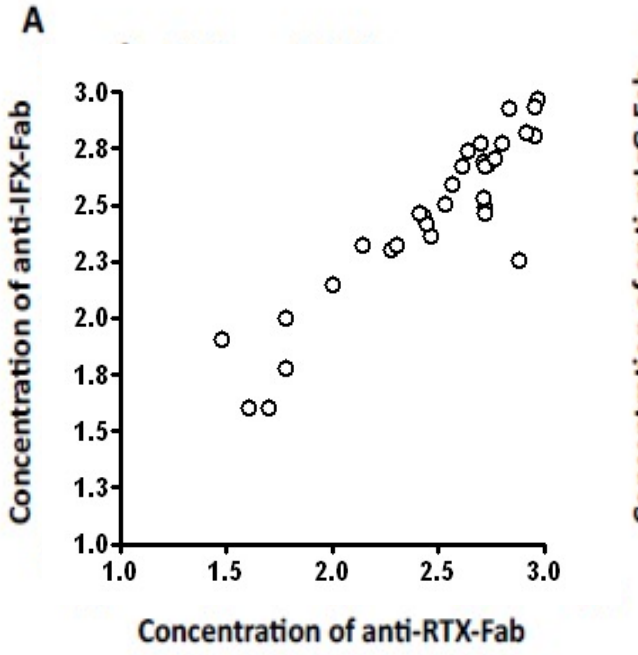


T-helper cells



Anti-mouse Fab, anti-IFX Fab and anti–RTX Fab correlate

r^2=0.90, p < 0.0001



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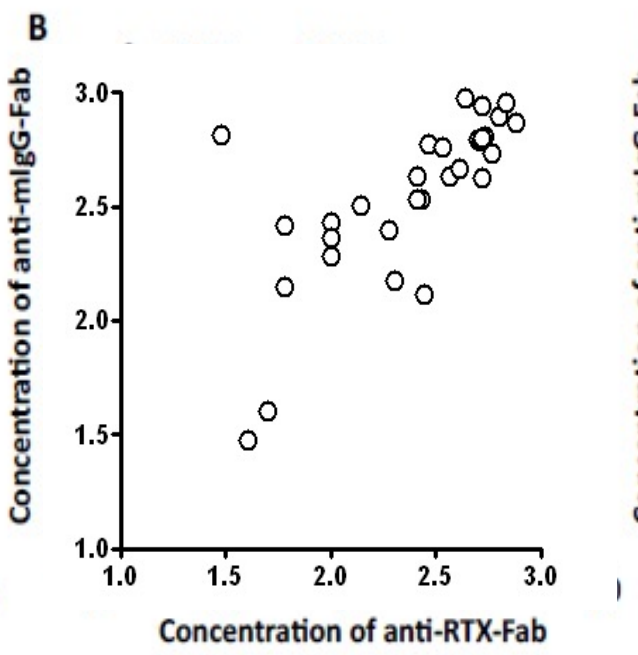

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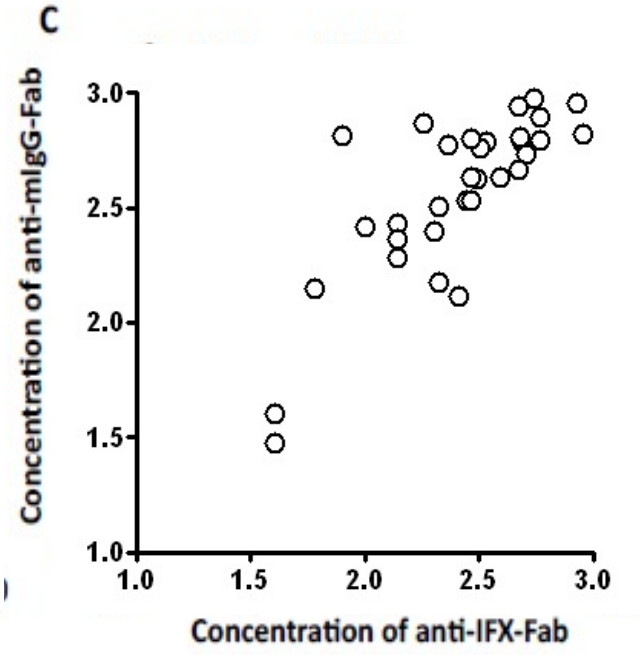
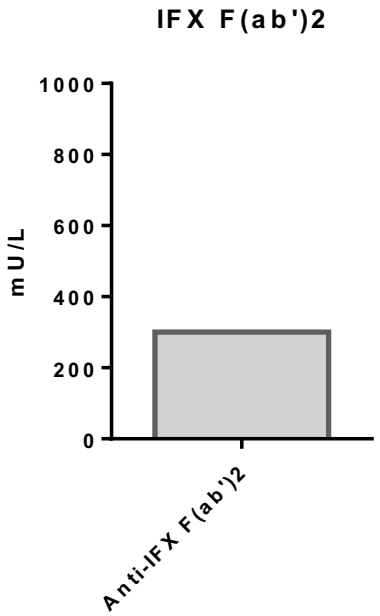
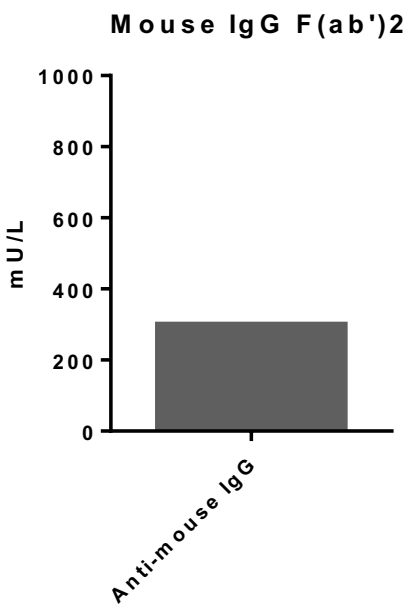


Cross-reactivity between anti-IFX and anti-mIgG

Mouse IgG column

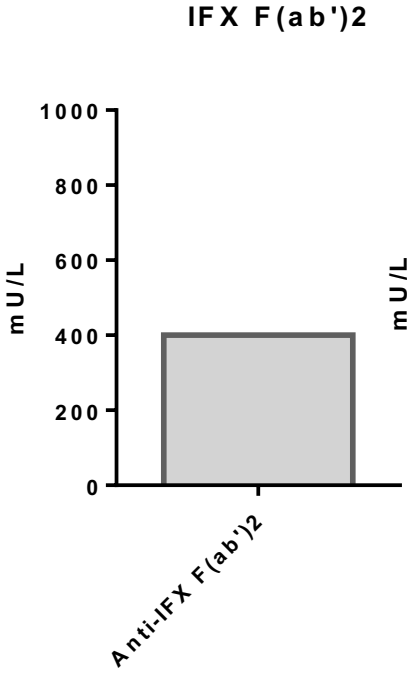


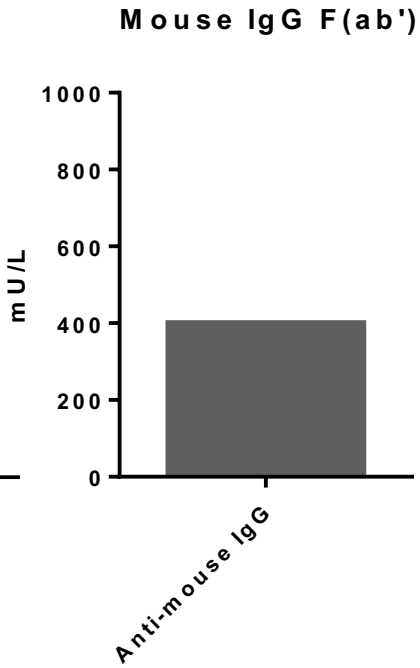




Infliximab column







AP&T Alimentary Pharmacology and Therapeutics

Pre-existing IgG antibodies cross-reacting with the Fab region of infliximab predict efficacy and safety of infliximab therapy in inflammatory bowel disease

C. Steenholdt*, Y. Palarasah†, K. Bendtzen†, A. Teisner‡, J. Brynskov*, B. Teisner† & C. H. Nielsen†

Aliment Pharmacol Ther 2013; 37: 1172–1183

Clinical study

Observational, retrospective, single center study

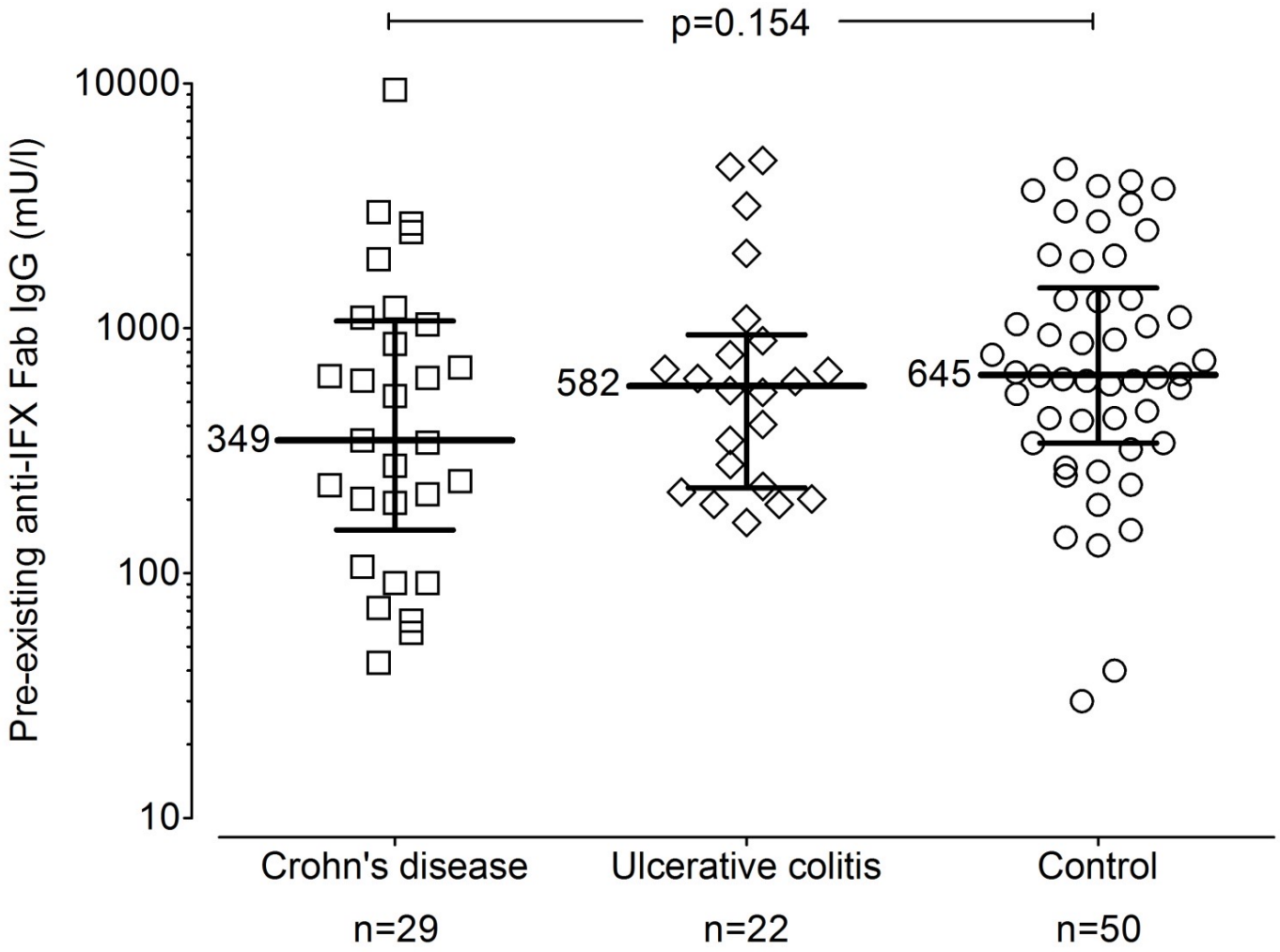
29 patients with Crohn's disease treated with infliximab

21 patients with ulcerative colitis treated with infliximab

Measurement of pre-existing anti-infliximab-Fab Abs
and clinical responses

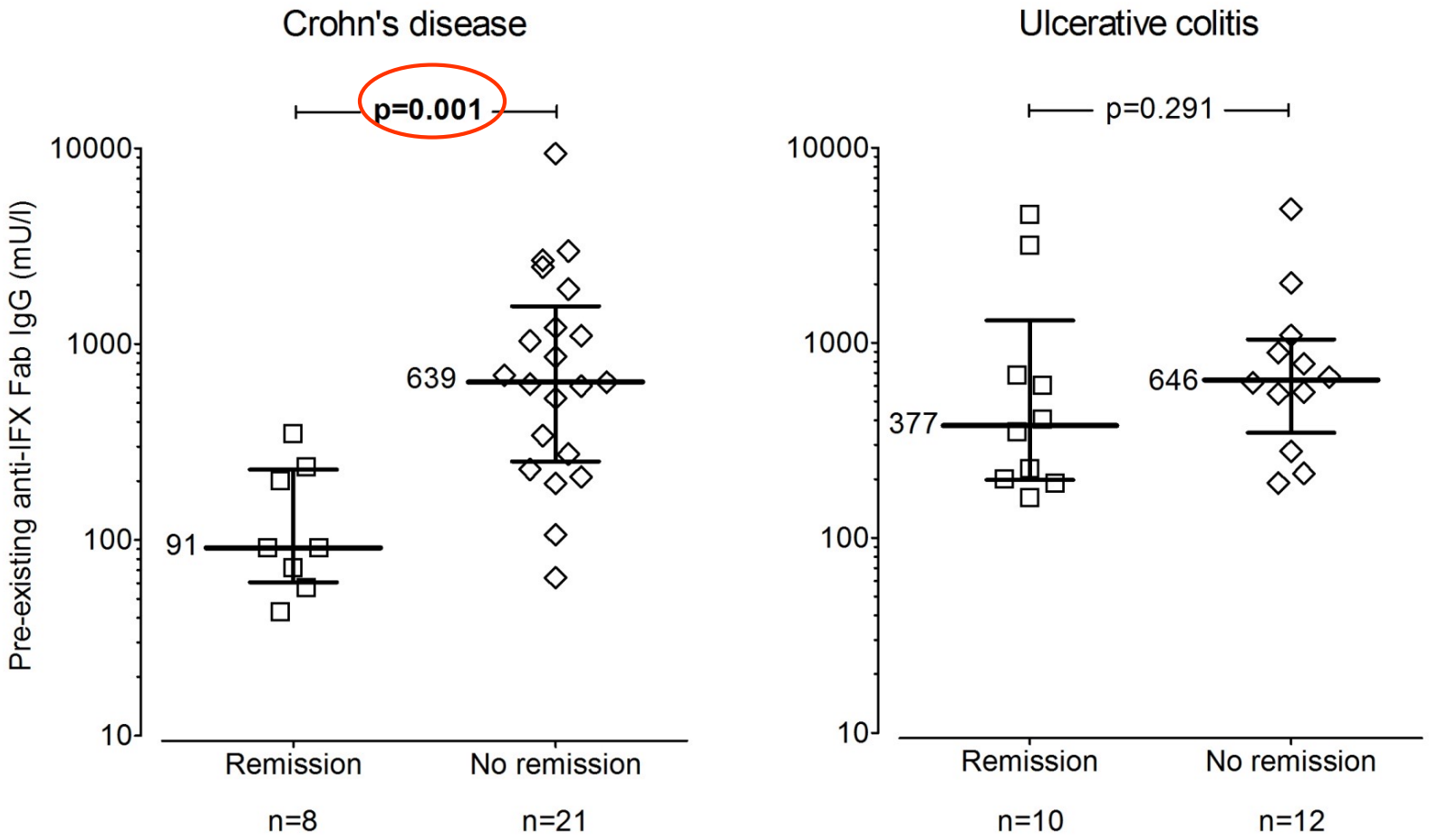
Steenholdt et al. Aliment Pharmacol Ther 2013; 37: 1172-83

Pre-existing anti-IFX Fab IgG: Prevalence in IBD patients naïve to 'biologics'



Steenholdt et al. Aliment Pharmacol Ther 2013; 37: 1172-83

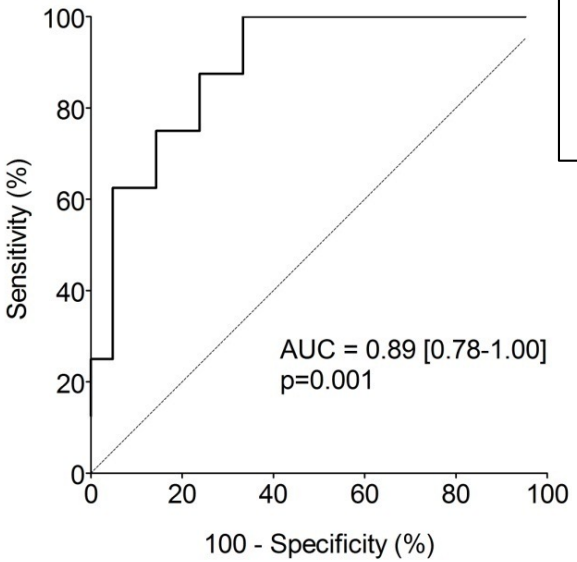
Pre-existing anti-IFX Fab IgG: Association with 1 year remission on IFX

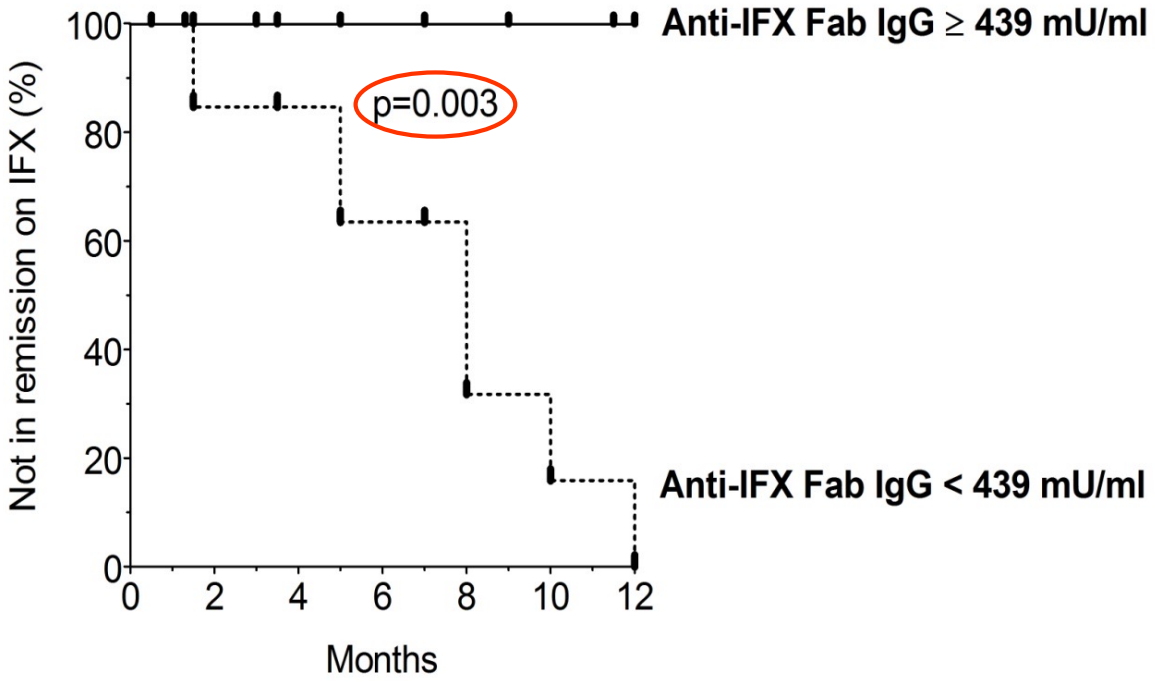


Steenholdt et al. Aliment Pharmacol Ther 2013; 37: 1172-83

Pre-existing anti-IFX Fab IgG: Levels associated with 1 year remission in CD

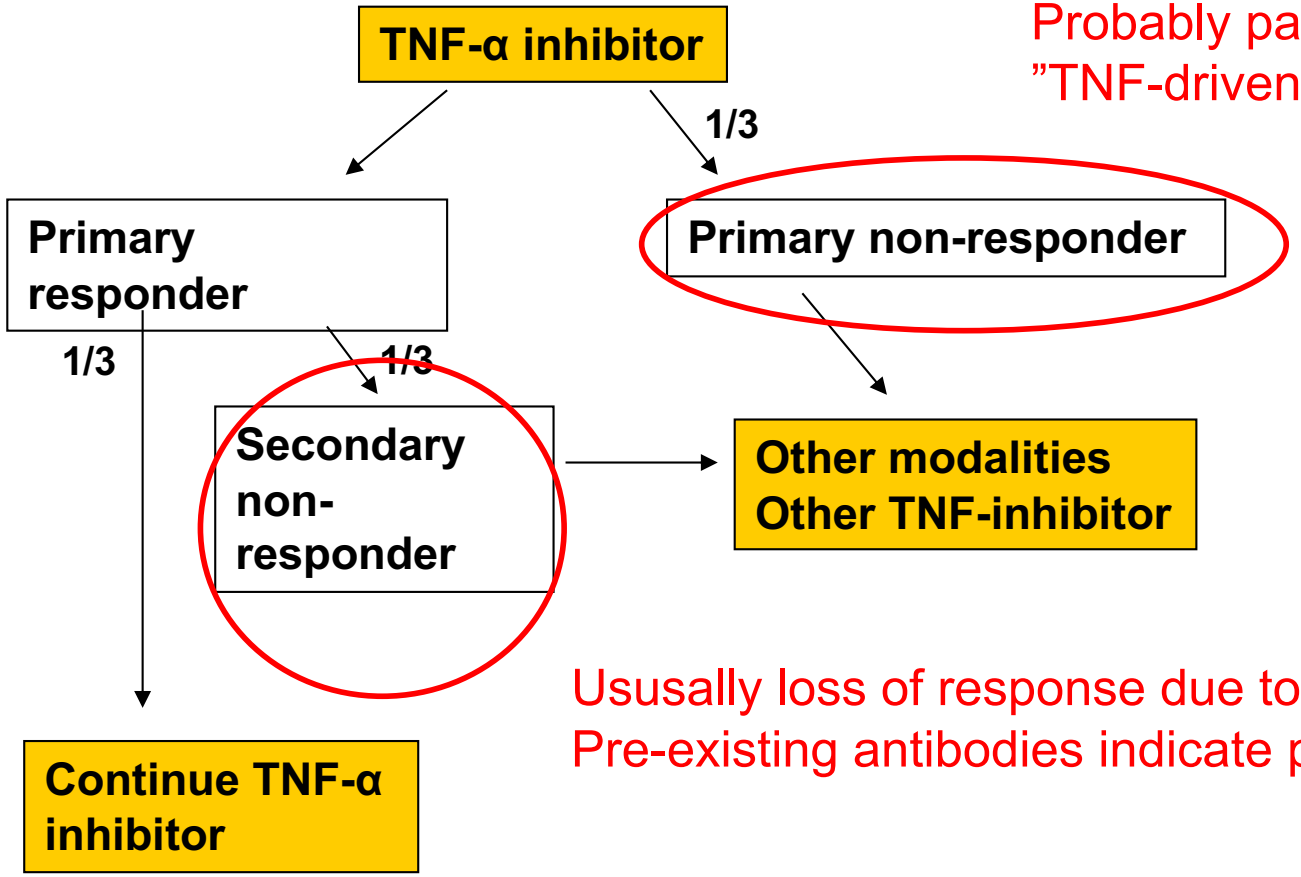
	Anti-IFX Fab Ab cut-off mU/l	Sensitivity % [95%CI]	Specificity % [95%CI]
Identification of patients **in remission**	<439	100 [63–100]	67 [43–85]
Identification of patients **not in remission**	≥61	100 [84–100]	25% [3–65]
Optimal differentiation between patients in remission or not (**sensitivity=specificity**)	233	75 [35–97]	76 [53–92]





Steenholdt et al. Aliment Pharmacol Ther 2013; 37: 1172-83

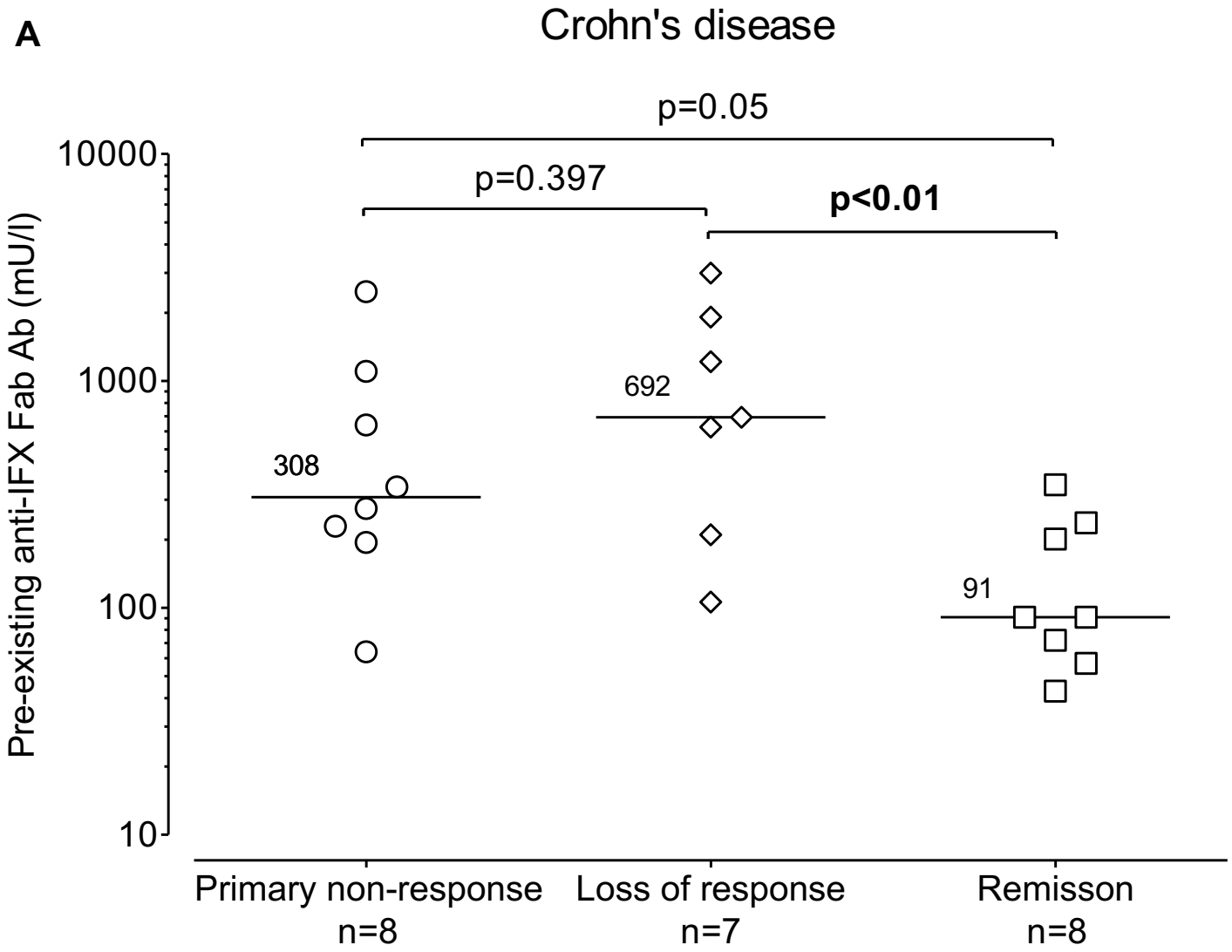
Primary vs. secondary non-responders



Probably patients without ”TNF-driven disease”

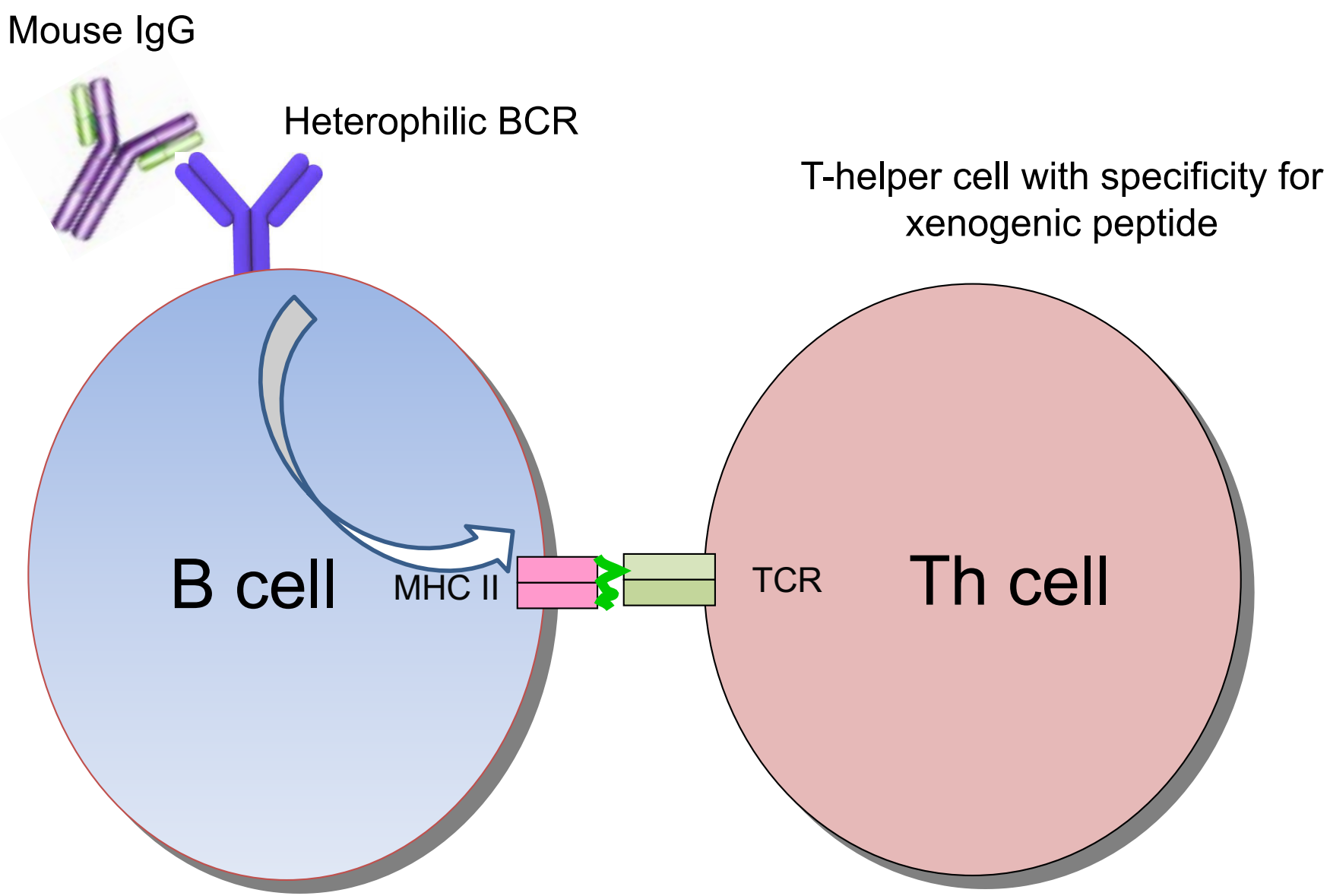
Ususally loss of response due to ADAs
Pre-existing antibodies indicate priming

Efficacy by anti-IFX Fab Abs



Steenholdt et al. Aliment Pharmacol Ther 2013; 37: 1172-83

Pre-existing T-cell responses may support development of drug-neutralizing antibodies



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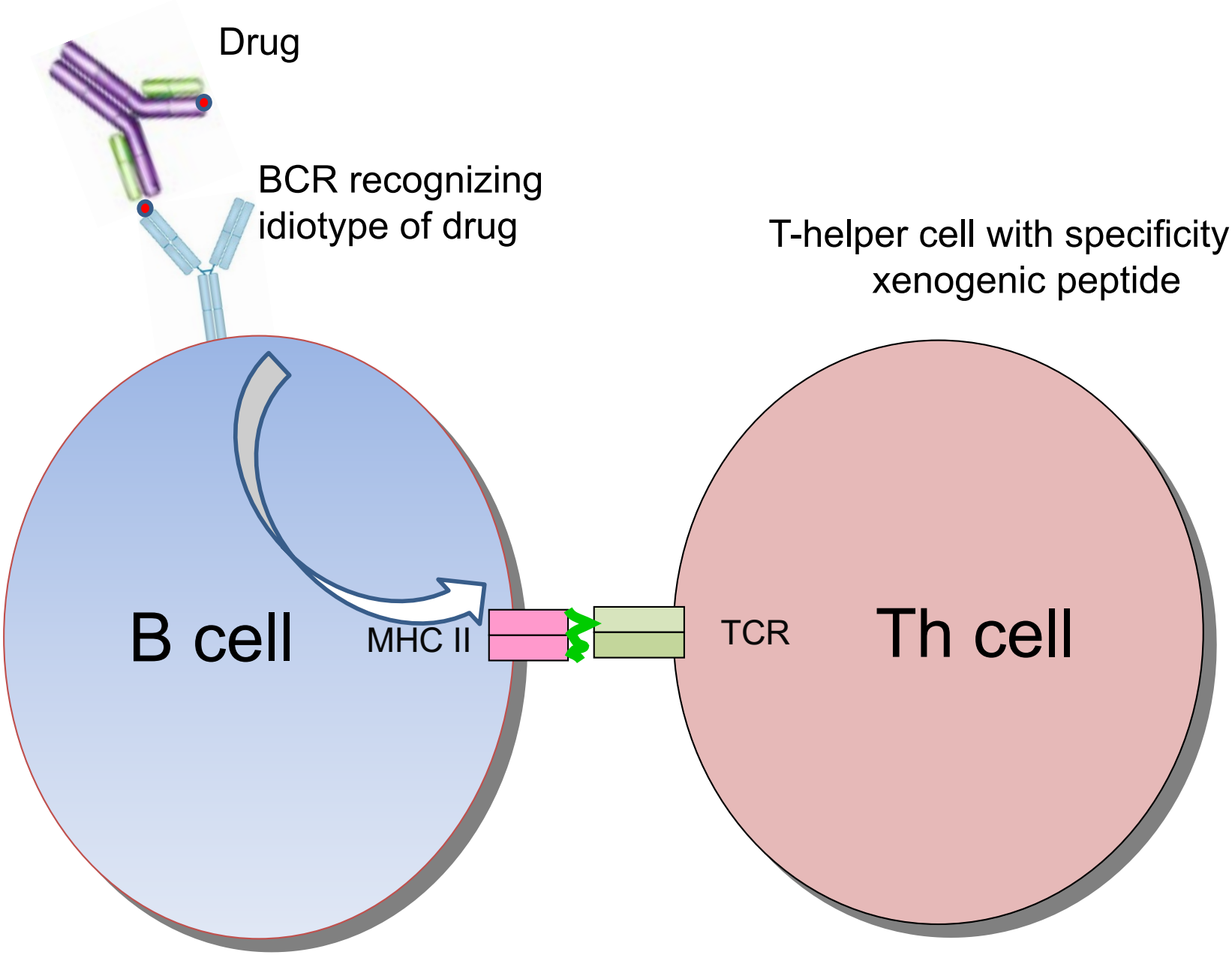
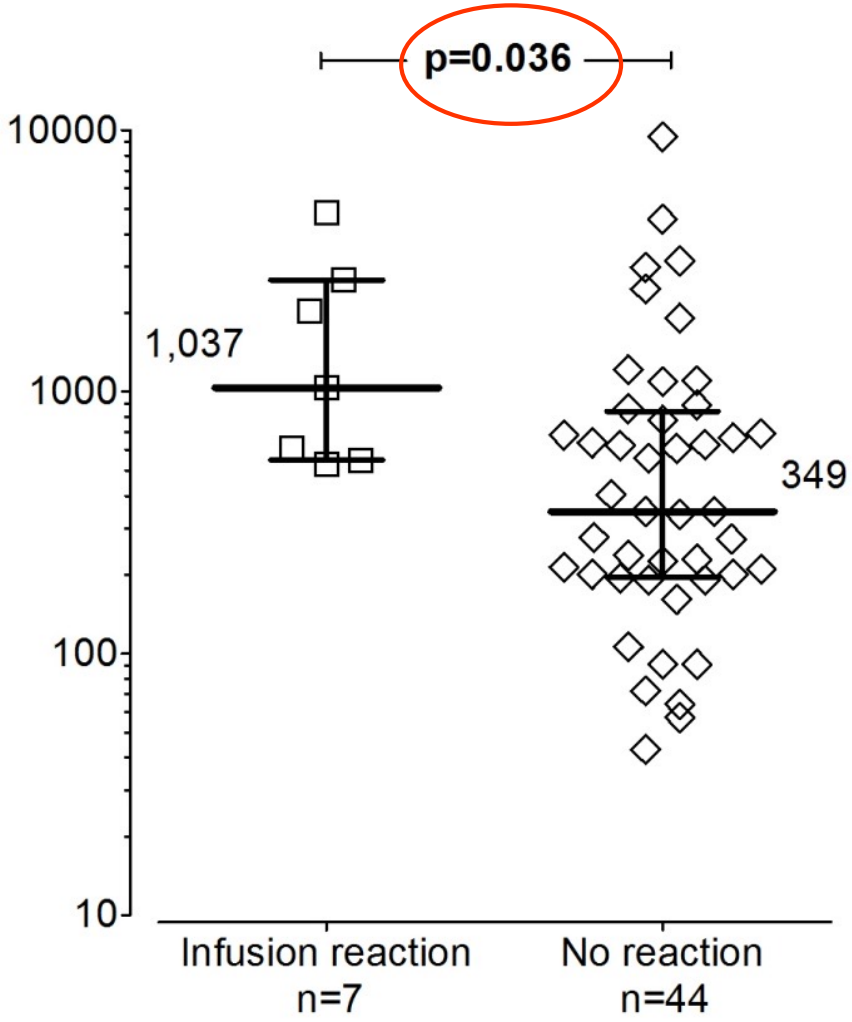


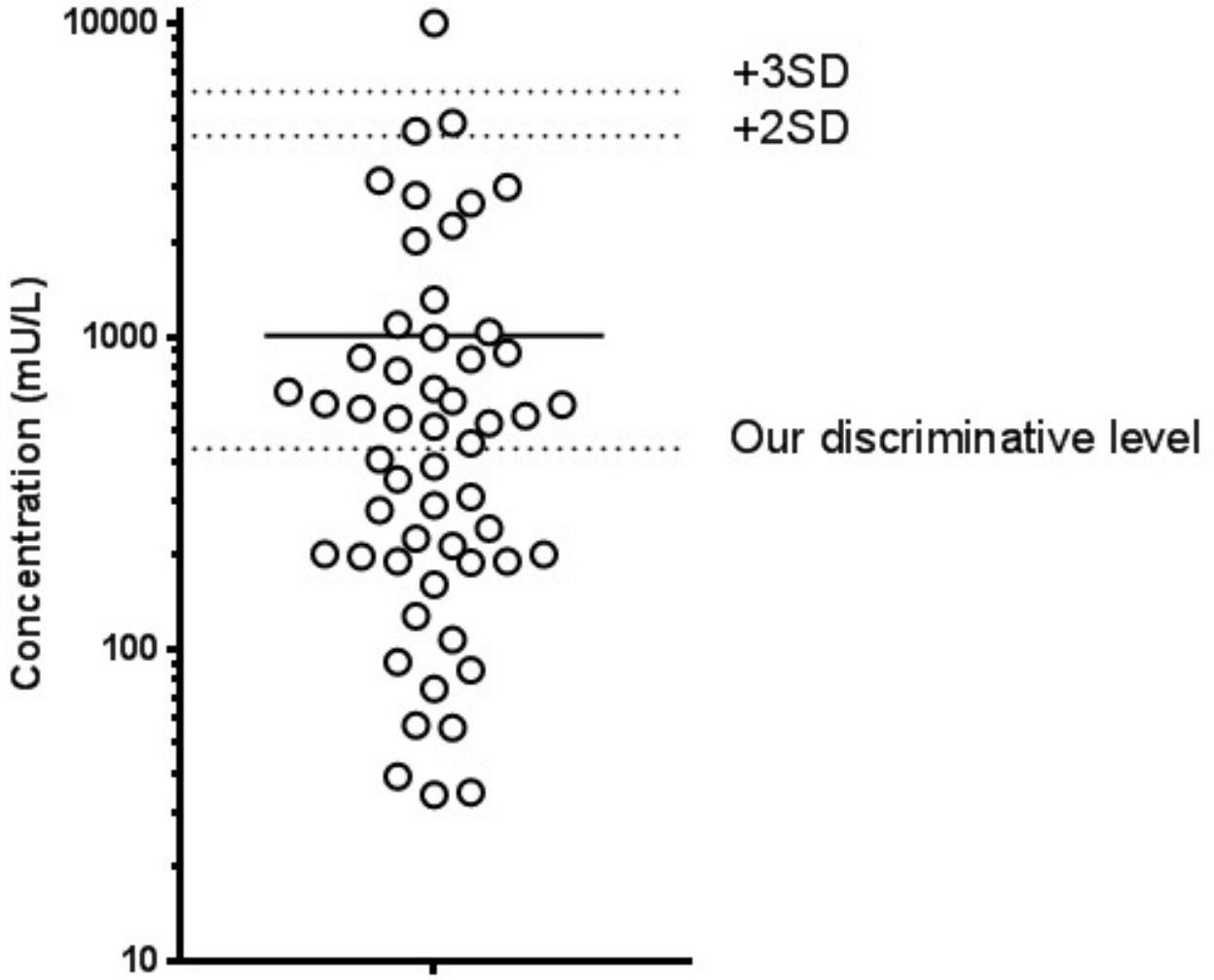
Safety by anti-IFX Fab Abs

All patients



Steenholdt et al. Aliment Pharmacol Ther 2013; 37: 1172-83

”Positive samples”



Meta-analysis by Xu & Rup

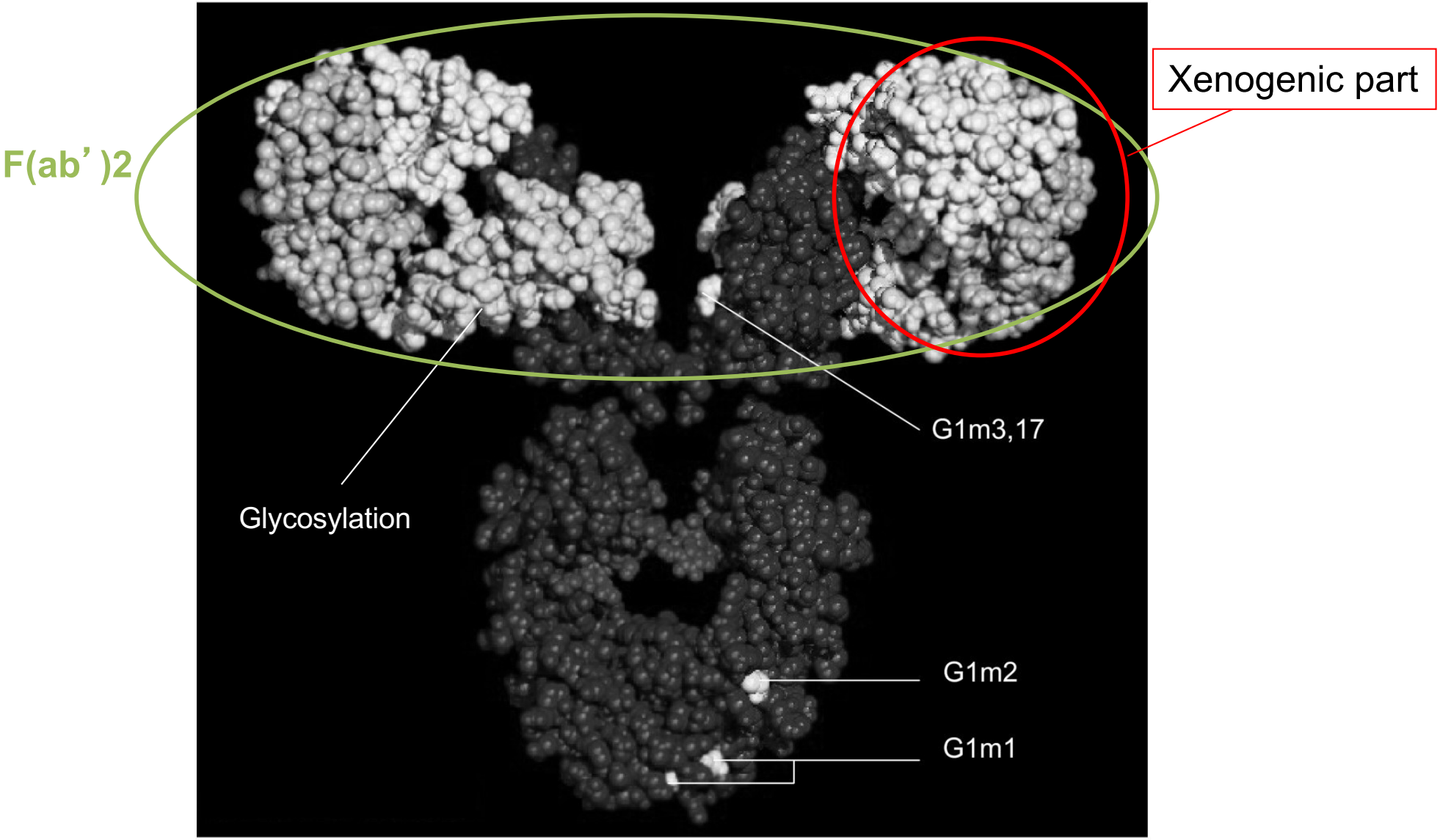
31 studies: 1331 patients & 499 controls

Table I. Analysis of Historical Clinical ADA Data for 12 Biotherapeutics

Product modality	Number of products	Study populations	Study phase	Number of studies	Number of study subjects	Prevalence of pre-existing Abs
Humanized mAb (IgG1)	6	Healthy volunteers, neurological disease, allergy, inflammatory disease, muscular dystrophy	Phases I, II	17	1,008	0–3.8%
Novel antibody-based constructs	3	Rheumatoid arthritis	Phase I	5	420	5.8–41.7%
Recombinant protein	2	Hemophilia, muscular dystrophy	Phases I, II	5	246	0–16.7%
mAb conjugate	1	Cancer	Phase I	4	156	1.9%

The AAPS Journal, Vol. 15, No. 3, July 2013 (© 2013)

What are the antigenic determinants?



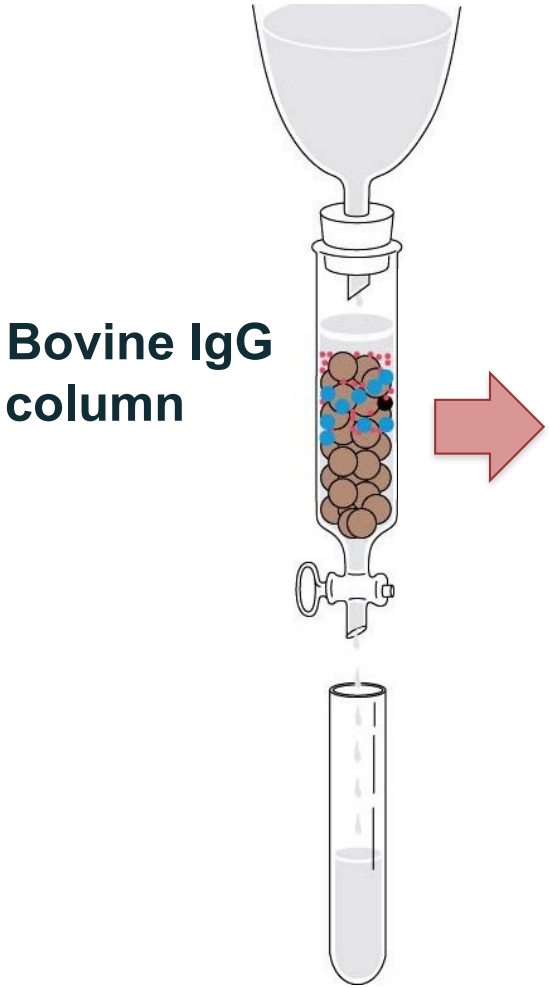
Origin of pre-existing antibodies?

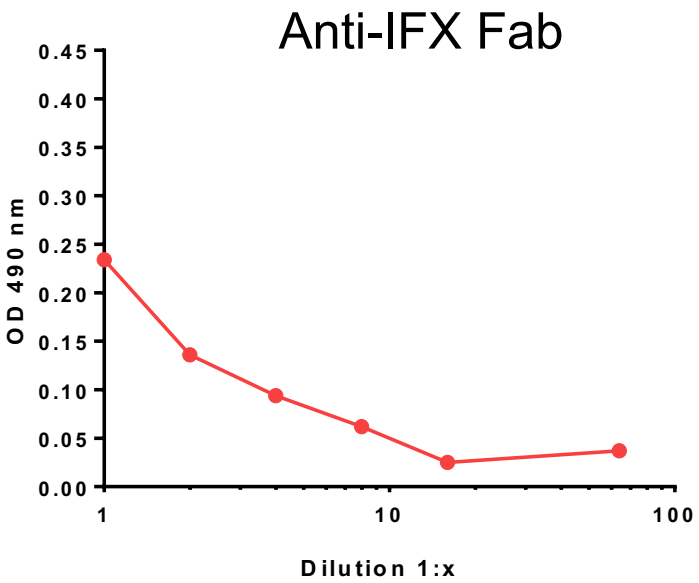
Heterophilic antibodies

Immunoglobulin	Group I Anti-Fab (85% of positive samples)	Group II Anti-Fc (15% of positive samples)	Negative samples
Mouse	+++	+++	-
Rat	+++	-	-
Goat	+++	-	-
Horse	+++	+++	-
Rabbit	-	+++	-
Cow	+++	+++	-
BSA (neg. Control)	-	-	-

Hennig et al. Journal of Immunological Methods 235 (2000) 71–80

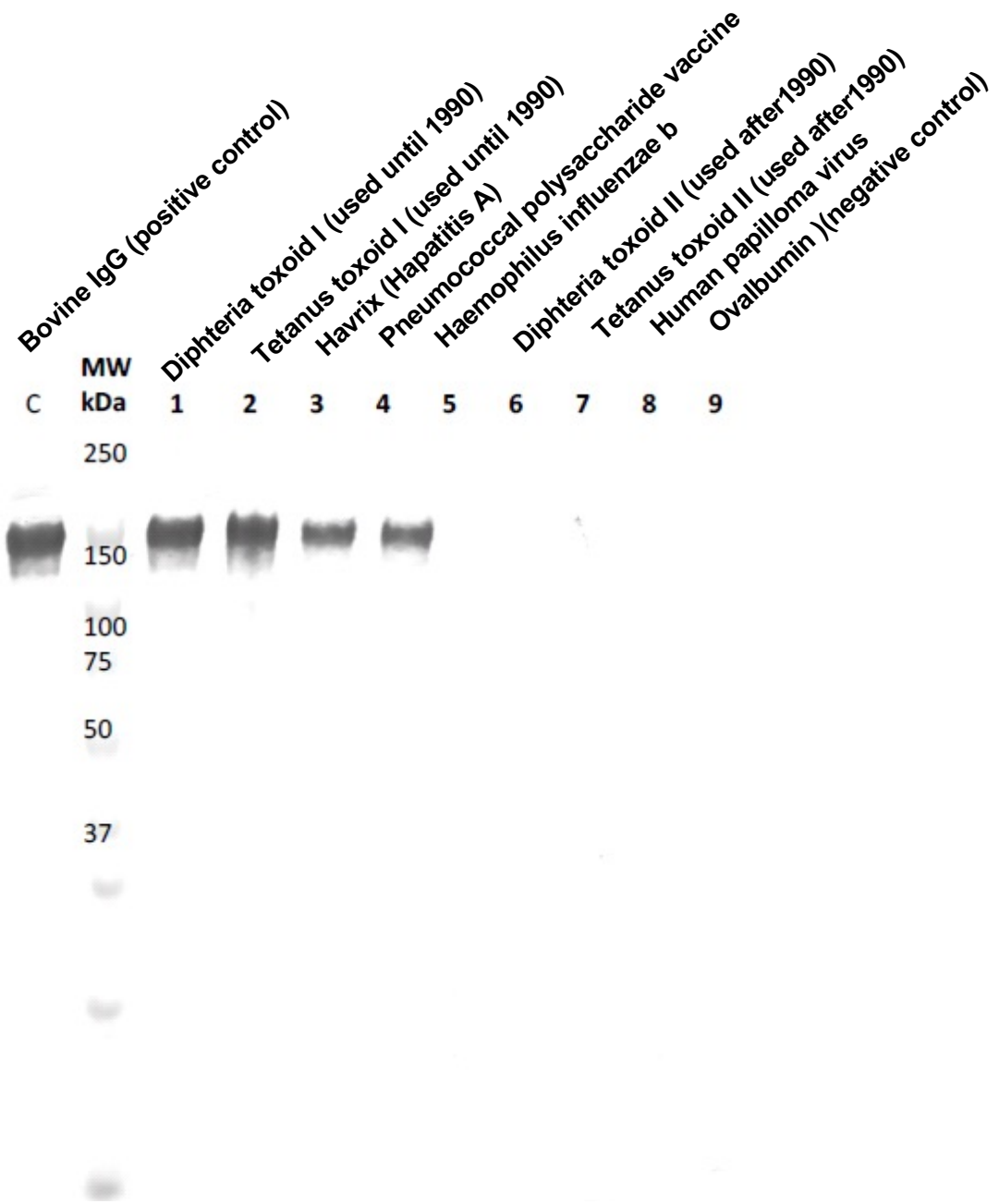
Cross-reactivity between cow IgG, mouse Fab and infliximab







Origin: Possibly vaccines



Conclusions regarding pre-existing antibodies

- exist against chimeric antibodies (IFX, RTX, CTX)
- are presumably ”heterophilic antibodies”
- apparently predict outcome of infliximab therapy in Crohn’s disease
- may cause IgG- or IgE-mediated infusion reactions
- may be induced by vaccination

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REGION H Rigshospitalet

Thanks to:

Yaseelan Palarasah, Christina Aniol-Nielsen, Casper Steenholdt, Børge Teisner

Everybody at Institute for Inflammation Research

