

## **IMMUNOGENICITY TESTS:**

# **Evaluation of the formation of antibodies against macromolecules**

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## Definitions

**Immunogenicity:** “The ability of an antigen or vaccine to stimulate immune responses”

**Macromolecules:** proteins, DNA, RNA, carbohydrates, complex solutions like surfactants

## **Guidelines on immunogenicity**

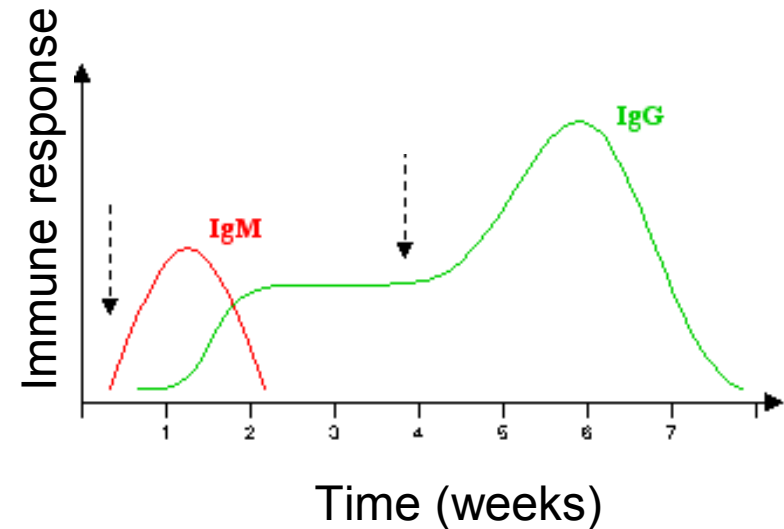
***ICH topic S6 - Preclinical safety evaluations of biotechnology-derived pharmaceuticals. CPMP/ICH/302/95***

***EMA - Guideline on comparability of medicinal products containing biotechnology-derived proteins as active substances. EMA/CPMP/ 3097/02***

***AAPS Ligand Binding Assay Bioanalytical Focus Group – AR Mire-Sluis et al., Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. J. of Immunological Methods (289, June 2004, p1-16)***

# Antibodies

- IgM:** Primary immune response
- IgG<sub>x</sub>:** Secondary immune response
- IgE:** Protection against parasites, activates allergic reaction
- IgD:** Found in blood stream; function unknown
- IgA:** Found in saliva, tears; not in the bloodstream



## Factors impacting the immunogenicity of a molecule

- Protein structure and size
- Formulation
- Impurities
- Route (i.v. / s.c. / i.m. /...) / dosis / frequency / duration of administration
- Genotype (MHC - major histocompatibility class)

## Potential clinical risks of immunogenicity

- Neutralizing antibody
  - altered PK/PD parameters leading to reduced or increased efficacy
  - altered PK/PD parameters leading to increased toxicity
  - Autoimmune disease
- Antibody mediated toxicity
- Anaphylactic responses

# **Analysis of antibodies**

## Antibody analysis

- Selection of antibodies to be measured
- What kind of assay is preferred?
  - Quantitative (continuous, calibration)
  - Relative quantitative (continuous, calibration)
  - Quasi-quantitative (continuous, analytical response units)
  - Qualitative (discrete response)

Assays should be *'fit for purpose'* !

## Quantitative antibody assays

Relative quantitative assay

- Assay with calibration curve

Quasi-quantitative assay

- Titer-based assay

Technique: ELISA

## Quantitative antibody assays

### Relative quantitative assay

- Assay with calibration curve

### Quasi-quantitative assay

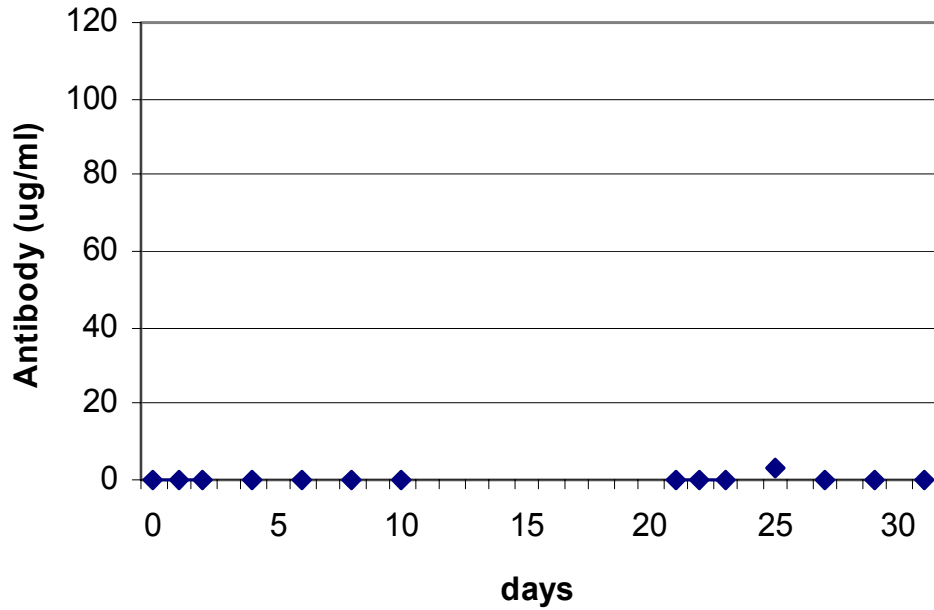
- Titer-based assay

➤ Availability of the -formed- antibody (reference material)

## Example

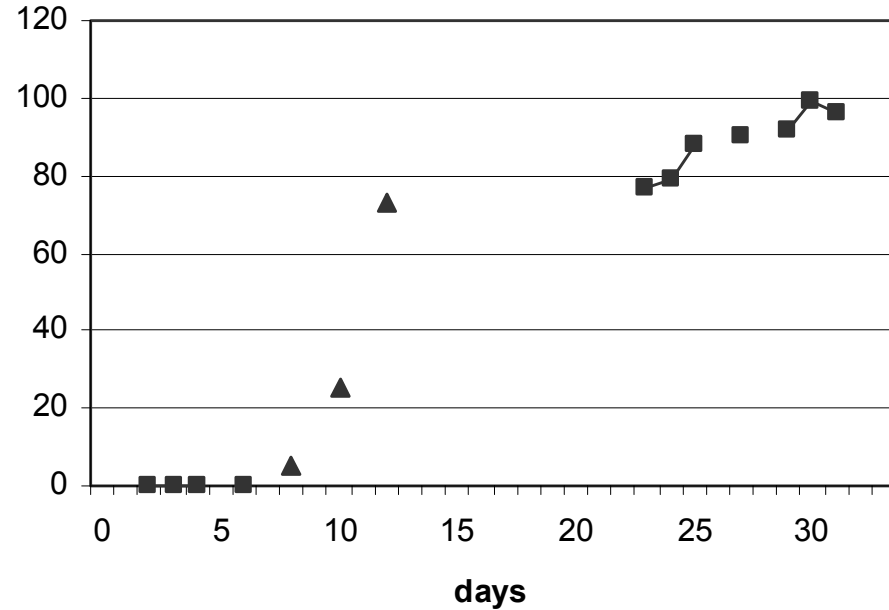
Calibrators / QC:	Commercially available antibody (mouse anti-human monoclonal)
Medication:	Human protein (modified)
Study:	Preclinical (Mouse and rabbit)
Purpose:	Determination of the IgG <sub>x</sub> antibodies against the modified human protein
Technique:	Direct ELISA

Rabbit



—◆— 75.0 mg/kg

Mouse



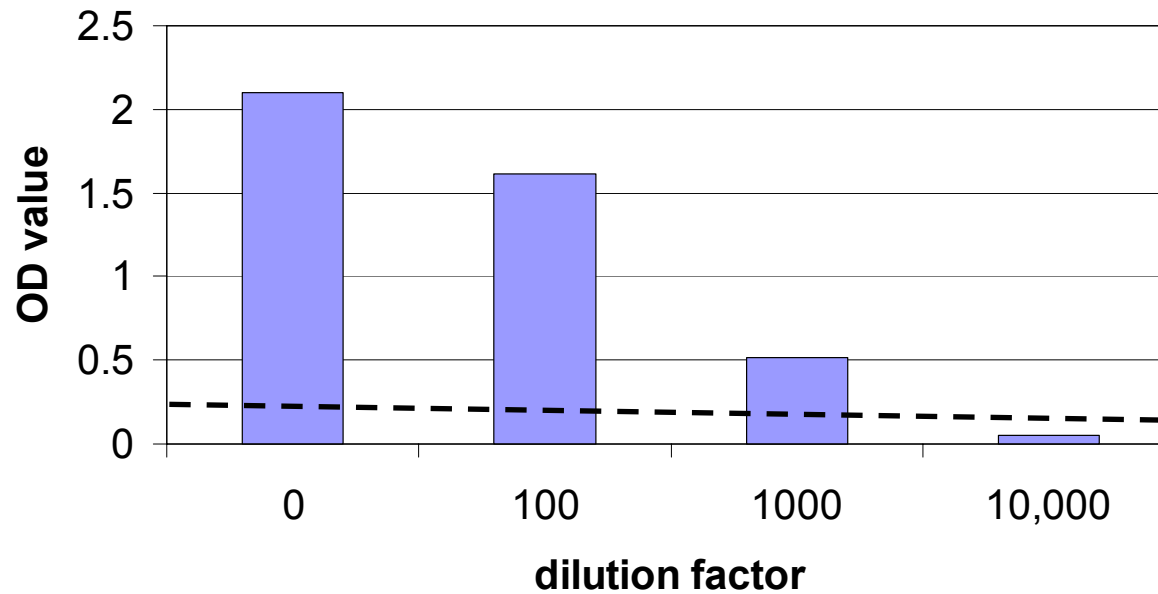
—■— 7.50 mg/kg  
—▲— 75.0 mg/kg

## Quantitative antibody assays

Relative quantitative assay

- Assay with calibration curve
- Quasi-quantitative assay
  - Titer-based assay

## Quasi-quantitative antibody assay



## Example: quasi-quantitative antibody assay

Cut-off:	2 x mean OD (3 healthy volunteers)
QC:	positive – rabbit anti-human antibody negative – serum from healthy volunteer
Medication:	Human protein (modified)
Study:	Clinical study (human patients)
Purpose:	Determination of the IgG antibodies against the modified human protein
Technique:	Direct ELISA

Subject	Sample	Cut-off	Mean OD	Normalized value	Score
1001	1	0.125	0.060	0.957	negative
1001	2	0.125	0.070	1.12	negative
1001	3	0.125	0.083	1.32	negative
1001	4	0.125	0.101	1.61	negative
1002	1	0.125	0.887	14.2	<b>positive</b>
1002	2	0.125	0.147	2.35	positive
1002	3	0.125	0.309	4.93	positive
1002	4	0.125	1.323	21.1	<b>positive</b>

Standard dilution-factor: 100x

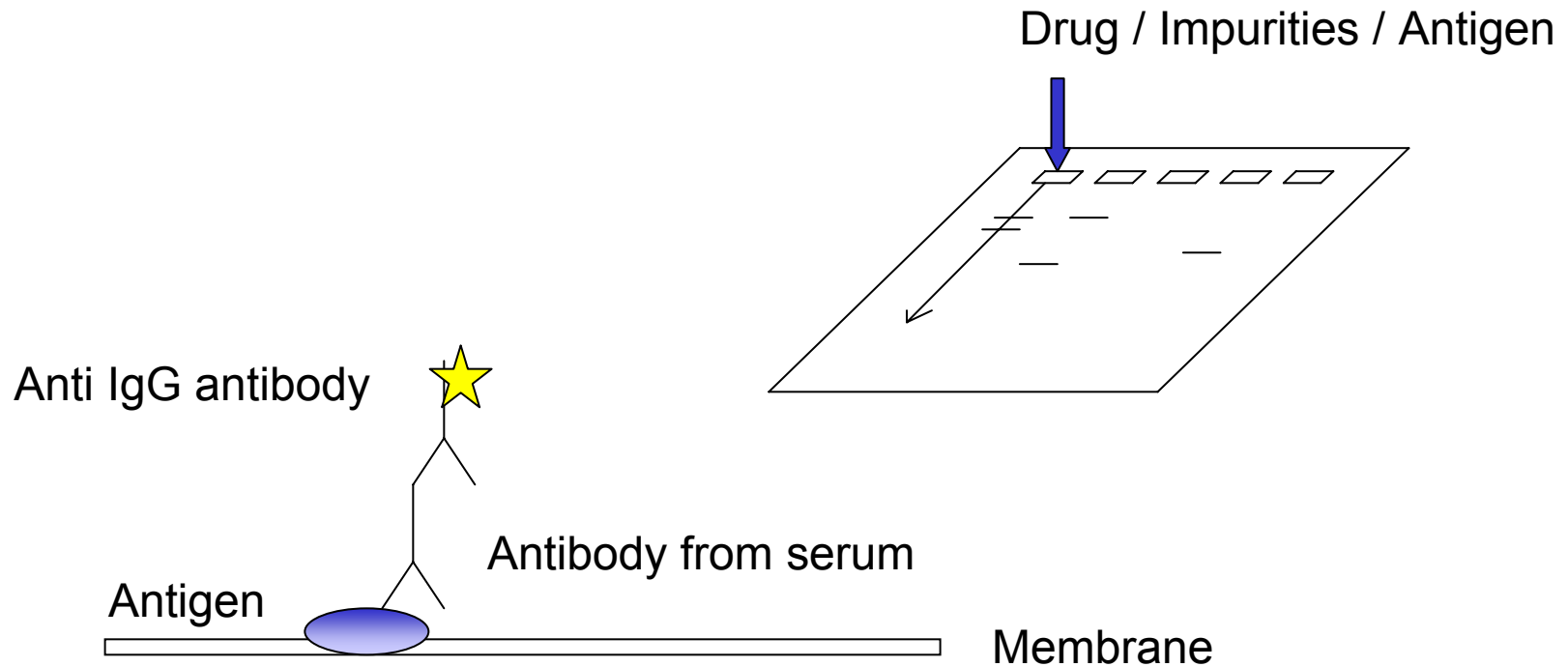
Subject	Sample	dilution factor (x 100)					
		4	8	16	32	64	128
1002	1	+	+	+	+	-	
1002	4	+	+	+	+	-	

## **Qualitative antibody analysis**

Target of immune response (what is the antigen?)

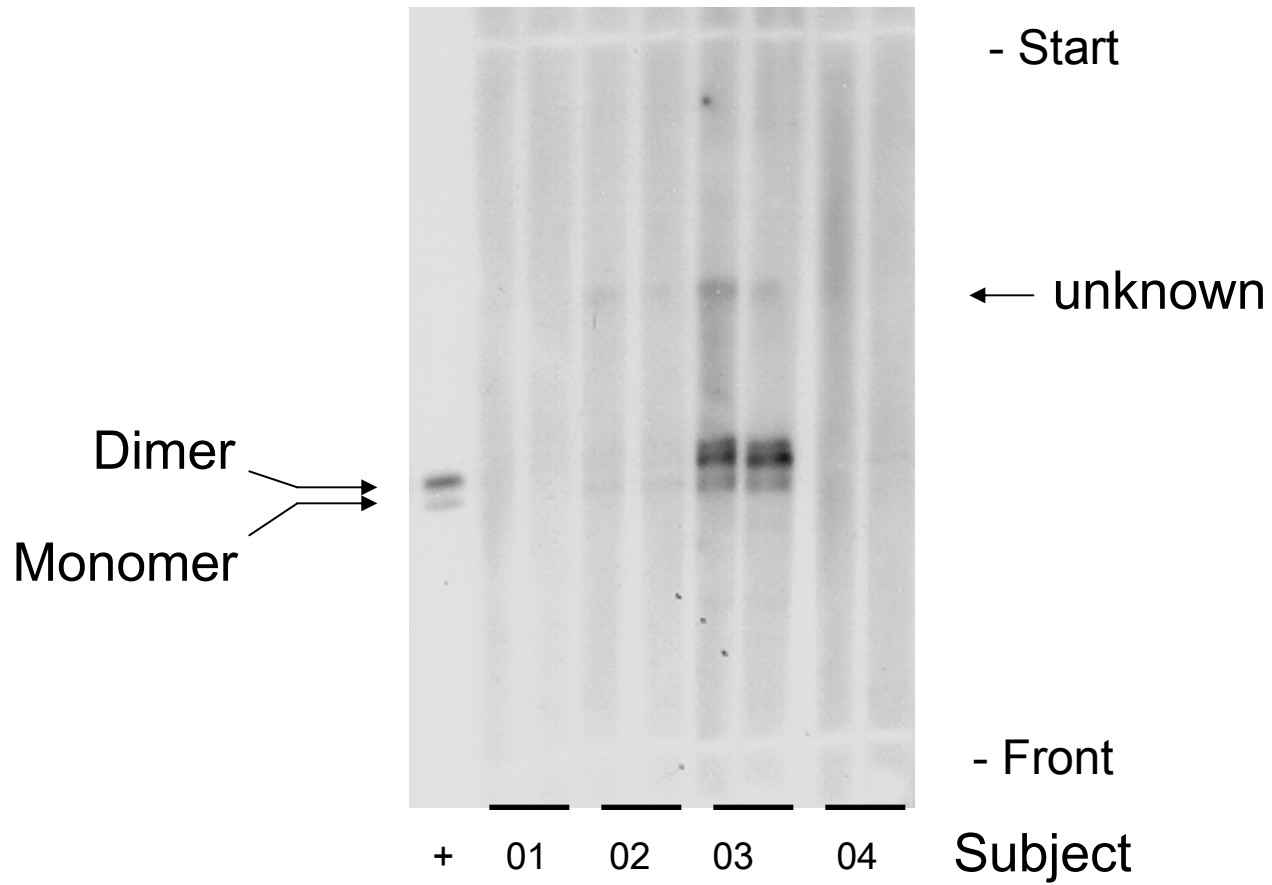
Technique: SDS-PAGE / Western Blot analysis

# Qualitative analysis of IgG using SDS-PAGE / Western Blot analysis



## Example

QC:	Antibody raised against a known impurity
Medication:	Human protein (modified)
Study:	Clinical study (healthy volunteers)
Purpose:	Determination of the antigen(s) in the medication



## Conclusions

- The immunogenicity of therapeutic proteins is a concern for clinicians, manufacturers and regulatory agencies.
- Different types of immunogenicity assays are available, each with its own characteristics and purpose.
- Immunogenicity tests should be carefully planned and evaluated (e.g. evaluation different species, population size, timing sample withdrawal, etc...)

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