



**New tools for therapeutic antibodies and anti-drug antibodies (ADA) detection in human serum**

**Background:** The therapeutic use of chimeric or humanized antibodies such as Remicade®, Mabthera® or Roactemra® has greatly improved the treatment of several autoimmune diseases (rheumatoid arthritis, psoriasis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, leukemias, or lymphomas). However, therapeutic failure is observed in a significant proportion of patients treated with therapeutic antibodies. Among several hypothesis, the development of an anti-drug response (Anti-drug antibody (ADA)) may explain the loss of response due either to degradation and/or to neutralization of the drug effect. Therefore, accurate monitoring of drug and anti-drug antibody levels should be implemented in routine clinical practices.

**Objective:** Development of a panel of murine monoclonal antibodies against Remicade®, Mabthera® or Roactemra® intended to monitor the level of therapeutic antibodies and anti-drug antibodies in patient's sera.

**Materials and Methods**

**Therapeutic antibodies used as immunogen**

Therapeutic antibody	Alias	Target	Description	Supplier
Remicade	Infliximab	TNF- $\alpha$	chimeric IgG1/ $\kappa$	Janssen BV
Mabthera	Rituximab	CD20	chimeric IgG1/ $\kappa$	Roche
Roactemra	Tocilizumab	IL6-R	humanized IgG1/ $\kappa$	Roche



Langerin

SEMA-6

## Immunization

The immunization program was performed according to Dendritics routine process.

Briefly, one BALB/c Jico (Charles River's Laboratory) was immunized with Remicade, Mabthera and Roactemra according to the following schedule:

- Day 0 : IP1 3X30µg mAb in complete Freund adjuvant (1ml)
- Day 14 : IP2 3X30µg mAb in incomplete Freund adjuvant (1ml)
- Day 21 : IP3 3X30µg mAb in PBS (1ml)
- Day 29 : IV 50µg pool 3 mAbs in PBS (0.5ml)+ IP 40µg pool 3 mAbs in PBS (0.5ml)
- Day 32 : Splenocytes isolation and fusion with SP2/0 myeloma cells.

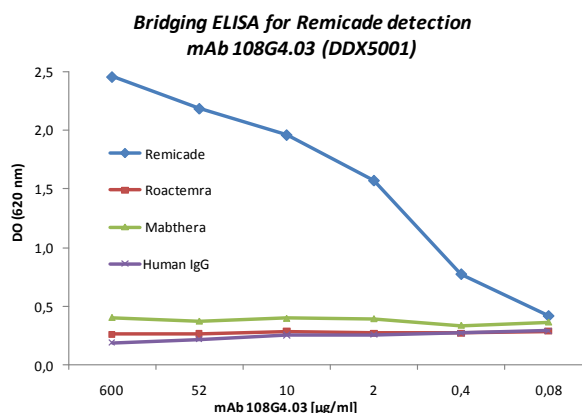
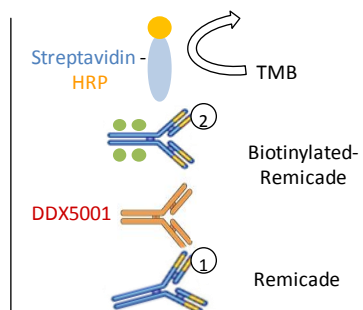
## Results

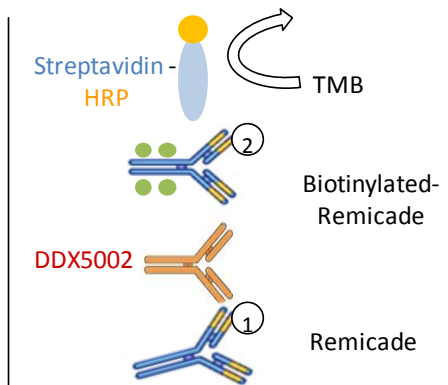
A panel of murine monoclonal antibodies was selected for each therapeutic antibody. These antibodies were set up in ELISA aiming at the dosage of both therapeutic antibodies and anti-drug antibodies (ADA) in human serum samples.

## REMICADE (DDX5001-DDX5002)

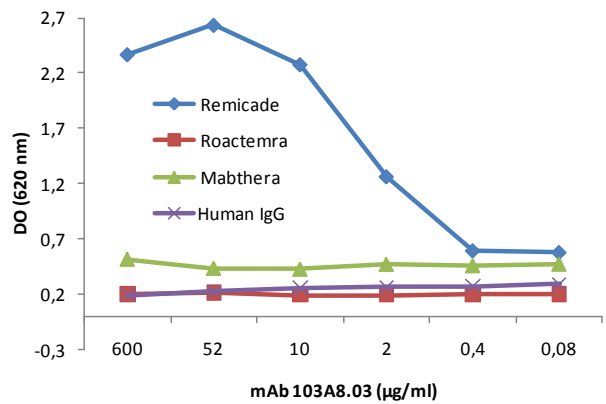
Remicade® is a chimeric monoclonal antibody targeting TNF- $\alpha$ , used for the treatment of autoimmune diseases such as rheumatoid arthritis, ankylosis spondylitis, Crohn's disease, psoriatic arthritis or ulcerative colitis. We have generated a panel of anti-Remicade® monoclonal murine antibodies. These antibodies can be used for the determination of Remicade® levels in biological fluids. In addition, competitive ELISA using these antibodies allows the dosage of anti-Remicade® in sera of patient under Remicade therapy.

### DDX5001 and DDX5002 are highly specific for Remicade

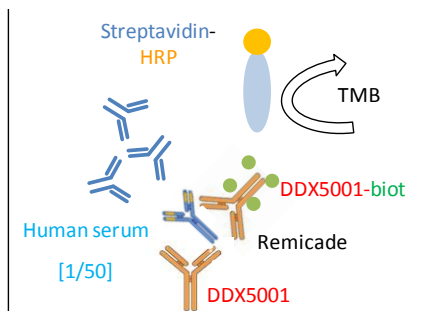




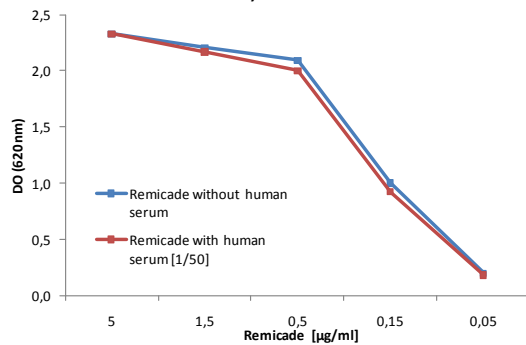
**Bridging ELISA for Remicade detection  
mAb 103A8.03 (DDX5002)**



**Remicade detection by DDX5001 is not affected by human serum**

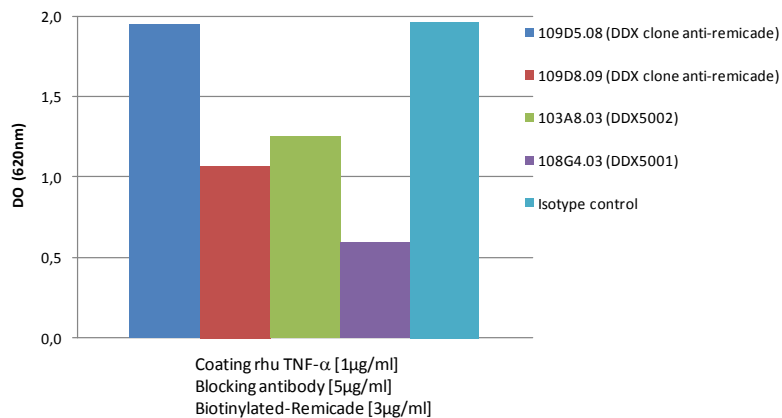


**ELISA detection of Remicade with or without human serum  
108G4.03/biotine-108G4.03**

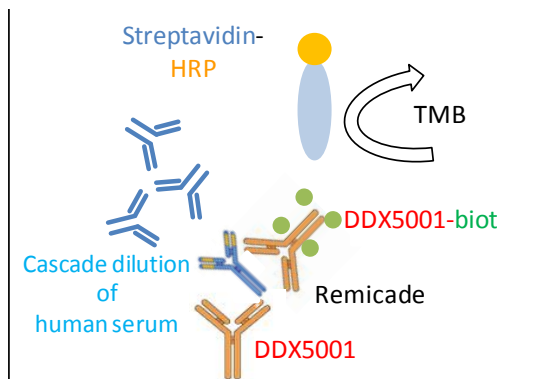


**DDX5001 and DDX5002 significantly inhibit Remicade binding to TNF-α**

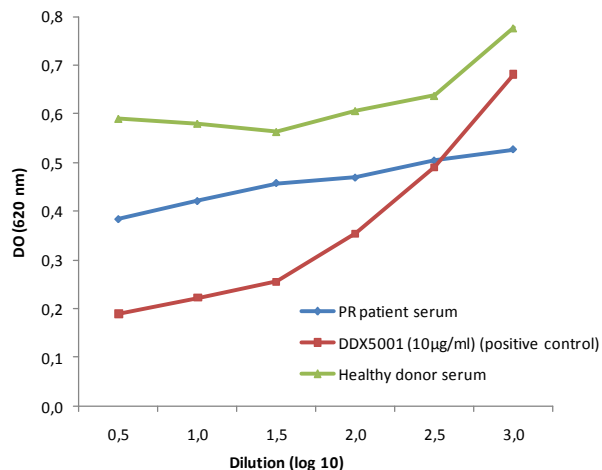
**Inhibition of Remicade binding to TNF-α**



## Anti-Remicade (ADA) detection by competitive ELISA in serum from Remicade-treated polyarthritis rheumatoid patient (PR)



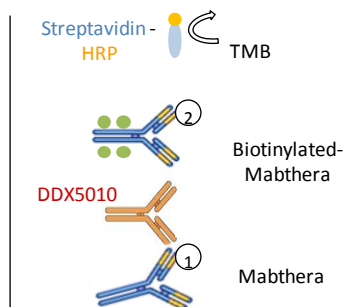
Detection of anti-Remicade (ADA) in human serum



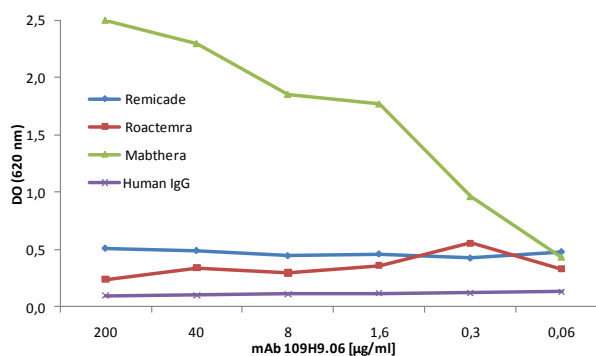
## MABTHERA (DDX5010-DDX5011-DDX5012)

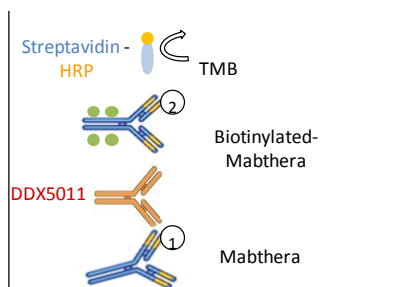
Mabthera® is a chimeric monoclonal antibody targeting the CD20 protein expressed on B cell surface. Mabthera® destroys B cells and is therefore used to treat diseases such as lymphomas, leukemias, transplant rejection, and autoimmune disorders. Mabthera® has also been shown to be effective in rheumatoid arthritis, in cases where anti-TNF- $\alpha$  therapy is not satisfactory. We have generated a panel of anti-Mabthera® monoclonal murine antibodies. These antibodies can be used for the determination of Mabthera® levels in biological fluids. In addition, competitive Elisa using these antibodies allows the dosage of anti-Mabthera® in patients sera.

## DDX5010 and DDX5011 are highly specific for Mabthera

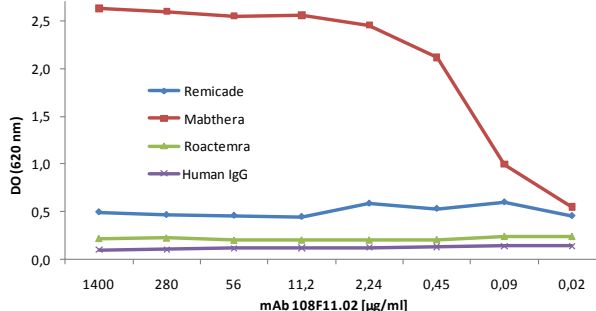


Bridging ELISA for Mabthera detection  
mAb 109H9.06 (DDX5010)

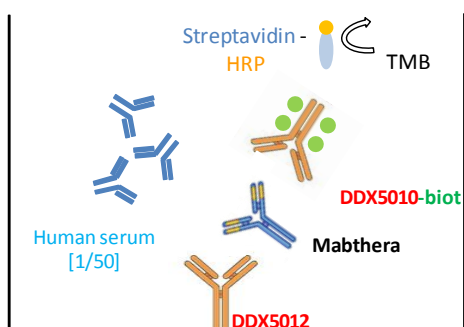




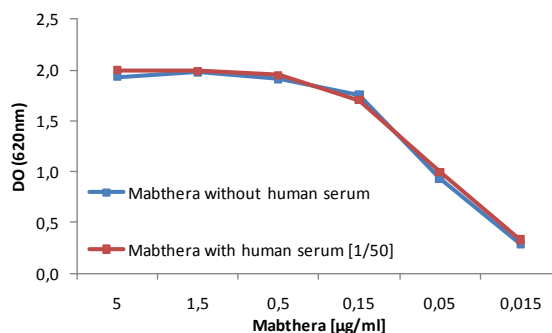
**Bridging ELISA for Mabthera detection  
mAb 108F11.02 (DDX5011)**



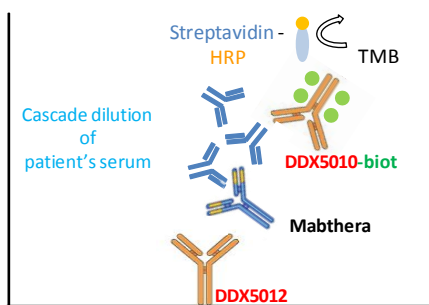
**Mabthera detection by DDX5010 is not affected by human serum**



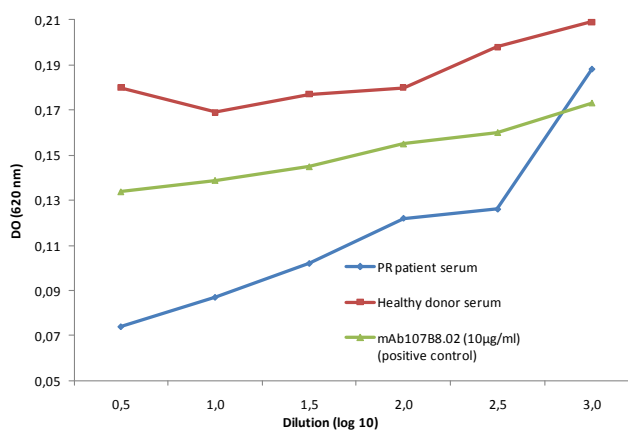
**ELISA detection of Mabthera with or without human serum  
DDX5010/DDX5010-biot**



**Anti-Mabthera (ADA) detection by competitive ELISA in serum from Mabthera-treated polyarthritis rheumatoid patient (PR)**



**Detection of anti-Mabthera (ADA) in human serum**

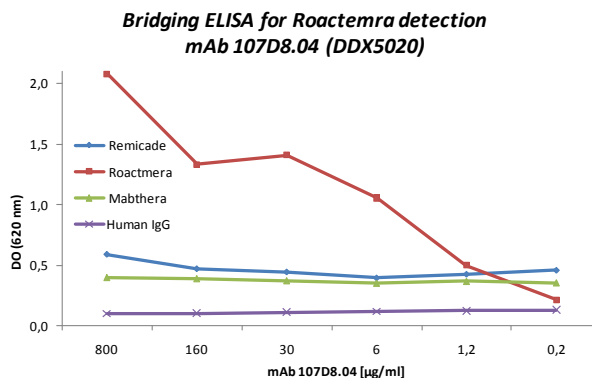
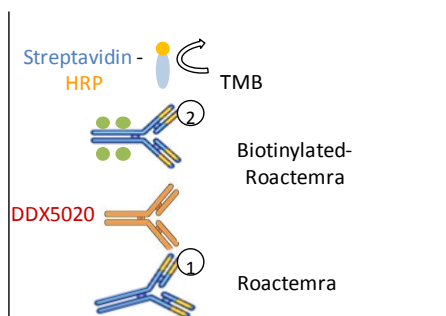


**ROACTEMRA (DDX5020-DDX5021)**

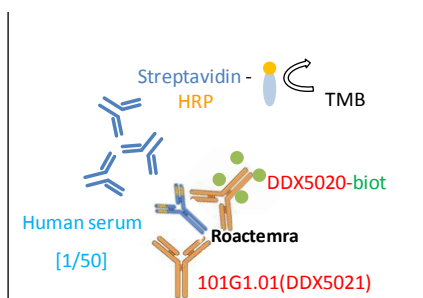
Roactemra® is a humanized antibody targeting the interleukine-6 receptor. This immunosuppressive drug is mainly used for the treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis. We have generated a panel of anti-Roactemra murine

monoclonal antibodies intended for Roactemra and anti-Roactemra (ADA) detection in patient's sera.

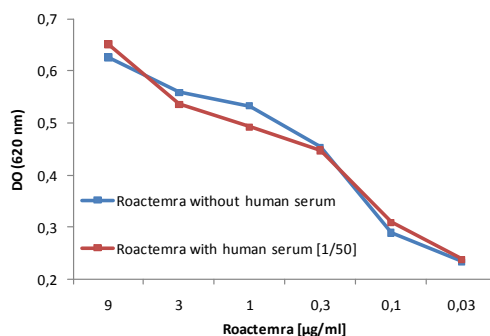
## DDX5020 is highly specific for Roactemra



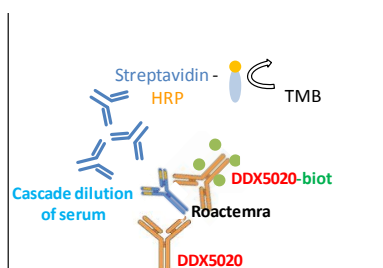
## Roactemra detection by DDX5020/101G1.01 is not affected by human serum



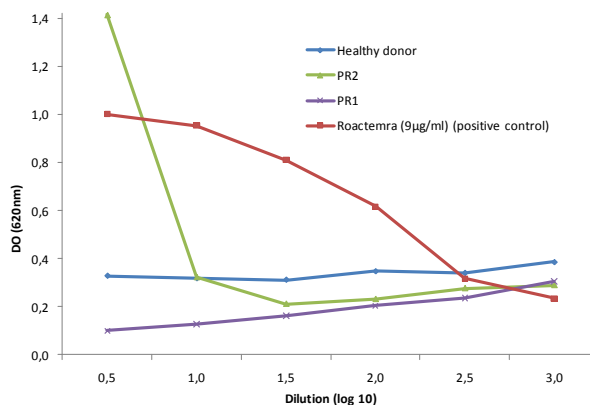
**Elisa detection of Roactemra with or without human serum  
101G1.01/DDX5020-biot**



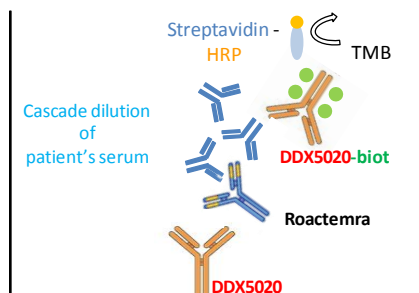
## Roactemra detection by capture Elisa in serum from Roactemra-treated polyarthritis rheumatoid patients



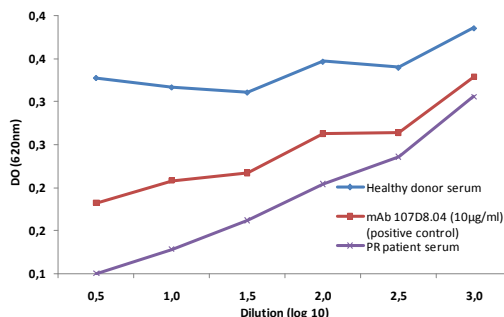
**Roactemra detection in PR patient's sera**



## Anti-Roactemra (ADA) detection by competitive ELISA in serum from Roactemra-treated polyarthritis rheumatoid patient (PR)



Detection of anti-Roactemra (ADA) in human serum



## SUMMARY TABLE

# catalog	Clone	Specificity			Detection	Capture	Bridging	Inhibition of remicade binding to TNF- $\alpha$	Isotype
		ROACTEMRA	REMICADE	MABTHERA					
	102C3.04	-	+	-	+	-	-	-	ND
	107A12.04	-	+	-	+/-	-	-	-	ND
	107C8.11	-	+	-	+/-	-	-	-	ND
	108D12.11	-	+	-	+	-	-	-	ND
<b>DDX5003</b>	108E3.11	-	+	-	+++	+	-	-	IgG1
	109D6.07	-	+	-	+/-	-	-	-	ND
	109G3.24	-	+	-	+++	+++	+	+/-	IgM
<b>DDX5004</b>	210D4.10	-	+	-	++	+++	-	-	IgG1
	210G4.02	-	+	-	+/-	-	-	-	ND
<b>DDX5001</b>	108G4.03	-	+	-	+++	+++	++++	++++	IgG1
<b>DDX5002</b>	103A8.03	-	+	-	+++	+++	++++	++	IgG1
	109D8.09	-	+	-	++	+++	++	++	IgG2a
	102B9.03	-	-	+	++	+++	+		
	107E9.03	-	-	+	++++	++++	+/-		
	107G12.06	-	-	+	+/-	-	-		
	107H12.02	-	-	+	++	+++	-		
	108G9.10	-	-	+	+++	+++	+		
	108H2.12	-	-	+	+++	++	-		
	108D1.23	-	-	+	+++	+++	+		
	108H5.02	-	-	+	++	+++	-		
	109A2.02	-	-	+	+	-	-		
	109B9.04	-	-	+	+	-	-		
	110D7.09	-	-	+	-	-	++		
	110B9.02	-	-	+	+++	+/-	+++		
	110F8.09	-	-	+	+/-	-	-		
	110F5.12	-	-	+	++++	++++	+++		
<b>DDX5010</b>	109H9.06	-	-	+	++++	+/-	++++		
<b>DDX5011</b>	108F11.02	-	-	+	+/-	-	+++		
<b>DDX5012</b>	107B8.02	-	-	+	++	++++	-		
	105D9.05	+	-	-	++	-	++		
	107E11.04	+	-	-	-	+/-	-		
	109C12.06	+	-	-	++	++	+		
	109B11.04	+	-	-	-	+/-	-		
	110A3.09	+	-	-	+++	++++	+		
	110E11.15	+	-	-	+++	++++	++		
	110H10.08	+	-	-	+/-	-	-		
	110E9.10	+	-	-	-	+/-	-		
<b>DDX5021</b>	101G1.01	+	-	-	++++	++	++		
<b>DDX5020</b>	107D8.04	+	-	-	++++	++++	+++		