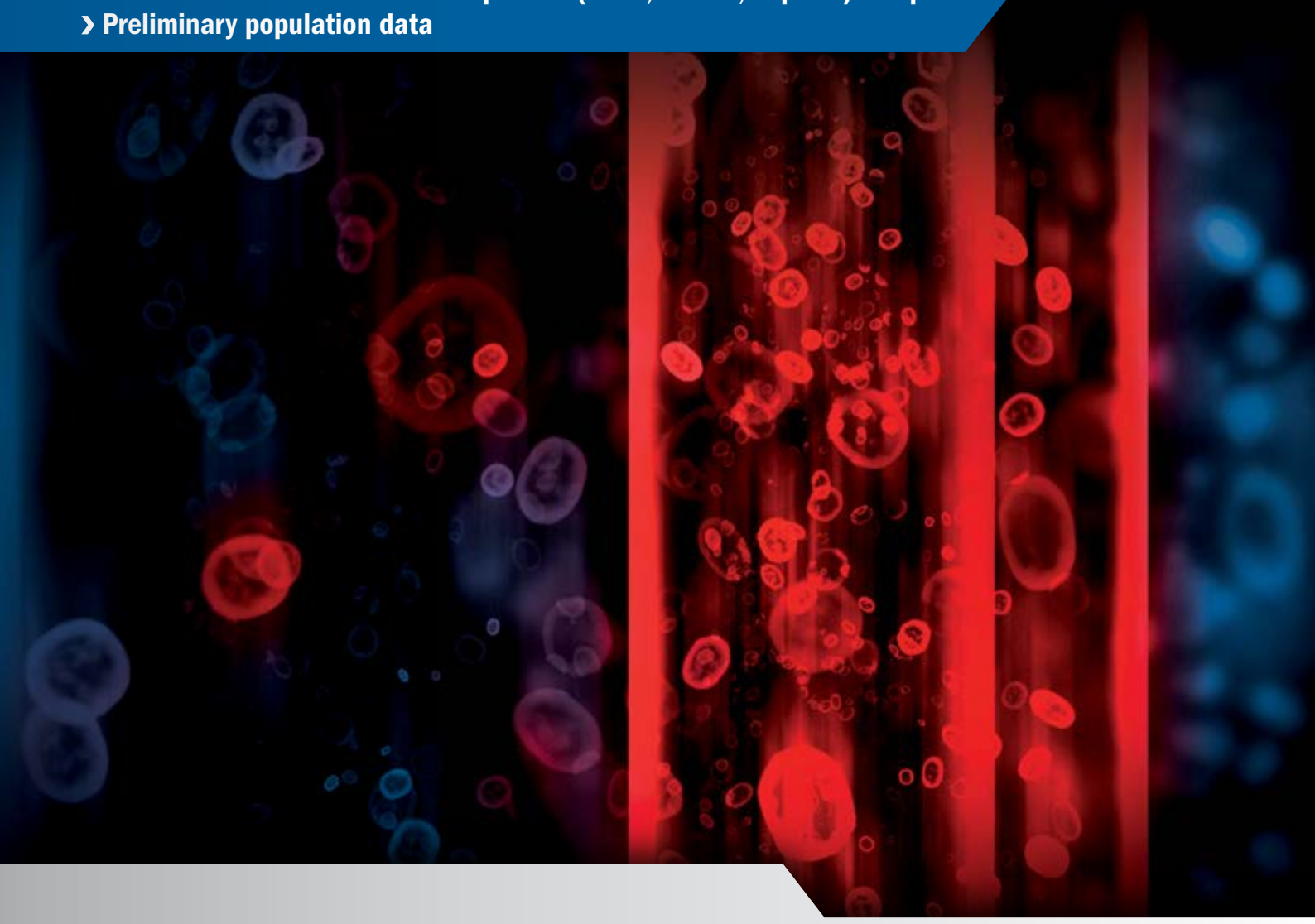
QUANTITATIVE DETERMINATION OF HUMAN ANTI-ALPHA-GALACTOSYL IgG, IgM, IgA "NATURAL ANTIBODIES"

NEW PRODUCT

Human Anti-Alpha-Galactosyl IgG ELISA
Human Anti-Alpha-Galactosyl IgM ELISA
Human Anti-Alpha-Galactosyl IgA ELISA

- Excellent analytical characteristics
- Validated for human serum and plasma (EDTA, citrate, heparin) samples
- Preliminary population data



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BioVendor
Research
and Diagnostic Products

HUMAN ANTI-ALPHA-GALACTOSYL IgG, IgM, IgA

Introduction

Natural antibodies circulate in serum of healthy humans naturally, without previous immunization [1]. They can be directed against the individual's own antigens as well as against foreign antigens [2, 3, 4]. The repertoire of natural antibodies is encoded by germ-line genes [5, 4, 6] and is not subjected to recombination. Natural antibodies are polyreactive [2, 3, 6] and react with low affinity but high avidity [7].

Among the natural antibodies directed against saccharide antigens, the best known are:
1) isohemagglutinins (anti-A, anti-B) which prevent the transfusion of allogeneic blood [8] and allotransplantation [9];
2) xenoagglutinins (80% of the repertoire of human xenoreactive natural antibodies is specific for the terminal α-galactose determinant) [10] which have traditionally been detected by agglutination test with rabbit or porcine erythrocytes [11]. The epitope against which they are directed was identified as Galα1-3Galβ1-4GlcNAc-R [12]. This epitope is present in cell membranes of mammals with the exception of humans and Old World primates [13] in which the gene encoding for α-1,3-galactosyltransferase was inactivated during evolution [14]. This epitope is also present in lipopolysaccharides and capsular polysaccharides of various gram-negative bacteria colonizing the gastrointestinal tract of humans [15].

In human serum, natural anti-α-galactosyl antibodies (anti-α-Gal) constitute approximately 4 - 8 % of total serum IgM, approx. 1 - 2 % of total serum IgG [16], and they can also be found as the IgA isotope in serum, saliva, milk, colostrum and vaginal washings [17, 15]. About 1 % of circulating B lymphocytes in adults is capable of producing these antibodies [18]. Their production is thought to be induced postnatally after antigenic stimuli by intestinal microflora [19].

The biological roles of natural antibodies are thought to be protection against infectious agents [20], particularly at the mucosal surface [15], effector functions of the immune system, e.g. in removing senescent or otherwise altered cells and physiologically degraded molecules [21], and regulation of immune responses, particularly autoimmune responses [6]. Moreover, the presence of anti-α-galactosyl antibodies in humans represents an important obstacle to xenotransplantations of porcine grafts since these antibodies participate in their rejection [22]. Natural antibodies also play an important role in malignant processes, and they have potential to be used in treatment of tumors that express these (α-Gal) antigens (e.g. breast cancer) [23, 24, 25, 26].

BioVendor Human Anti-Alpha-Galactosyl IgG ELISA (RD199178100R)
BioVendor Human Anti-Alpha-Galactosyl IgM ELISA (RD199178110R)
BioVendor Human Anti-Alpha-Galactosyl IgA ELISA (RD199178120R)

Intended use

The Human Anti-Alpha-Galactosyl IgG (or IgM or IgA, respectively) ELISA is an enzyme immunoassay for the quantitative measurement of human anti-alpha-galactosyl antibodies in the IgG (or IgM or IgA, respectively) class.

- The total assay time is less than 3 hours
- The kit measures IgG (or IgM or IgA, respectively) anti-alpha-galactosyl antibodies in human serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Calibrator is human serum based
- Components of the kit are provided ready to use, concentrated or lyophilized

Clinical application

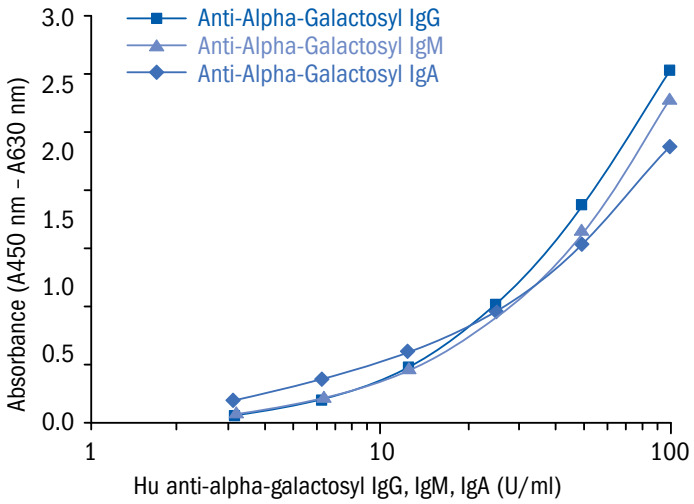
- Immunology, Immune Response
- Autoimmunity
- Infection and Inflammation
- Transplantation
- Oncology

QUANTITATIVE DETERMINATION OF HUMAN ANTI-ALPHA-GALACTOSYL IgG, IgM, IgA

Test principle

In the BioVendor Human Anti-Alpha-Galactosyl IgG (or IgM or IgA, respectively) ELISA, Calibrators and samples are incubated in microtitration wells pre-coated with chemically synthesized molecule displaying terminal beta-disaccharide Galα1-3Gal which is recognized by human anti-alpha-galactosyl antibodies. After 60 minutes incubation followed by washing, polyclonal antibody against human IgG (or IgM or IgA, respectively) conjugated with horseradish peroxidase (HRP) is added into the wells and incubated with the captured anti-alpha-galactosyl IgG (or IgM or IgA, respectively) for 60 minutes. The last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of IgG (or IgM or IgA, respectively) anti-alpha-galactosyl antibodies. A calibration curve is constructed by plotting absorbance values against anti-alpha-galactosyl IgG (or IgM or IgA, respectively) concentrations of calibrators and concentrations of unknown samples are determined using this calibration curve.

HUMAN ANTI-ALPHA-GALACTOSYL IgG ELISA CAT. NO.: RD199178100R	
Standards	3.13 to 100 U/ml
Limit of detection	1.44 U/ml
HUMAN ANTI-ALPHA-GALACTOSYL IgM ELISA CAT. NO.: RD199178110R	
Standards	3.13 to 100 U/ml
Limit of detection	1.35 U/ml
HUMAN ANTI-ALPHA-GALACTOSYL IgA ELISA CAT. NO.: RD199178120R	
Standards	3.13 to 100 U/ml
Limit of detection	0.61 U/ml



Summary of protocol

- Reconstitute Master Calibrator and prepare set of Calibrators
- Dilute samples (100x)
- Add 100 µl Calibrators and samples
- Incubate at RT for 1 hour
- Wash plate 3 times
- Add 100 µl Conjugate Solution
- Incubate at RT for 1 hour
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 30 min
- Add 100 µl Stop Solution
- Read absorbance and calculate results

HUMAN ANTI-ALPHA-GALACTOSYL IgG, IgM, IgA

Precision

Intra-assay (Within-Run) (n=8)

Sample	Coefficient of Variation (%)		
	Human Anti-α-Gal IgG ELISA	Human Anti-α-Gal IgM ELISA	Human Anti-α-Gal IgA ELISA
1	3.3	2.7	1.7
2	3.7	3.1	6.8

Inter-assay (Run-to-Run) (n=5)

Sample	Coefficient of Variation (%)		
	Human Anti-α-Gal IgG ELISA	Human Anti-α-Gal IgM ELISA	Human Anti-α-Gal IgA ELISA
1	5.1	8.4	5.7
2	7.9	4.4	8.0

Spiking recovery

Serum samples were spiked with different amounts of human anti-alpha-galactosyl IgG (or IgM or IgA, respectivelly) antibody and assayed. Results are expressed as observed/expected ratio (O/E) range.

ELISA	O/E Range (%)
Human Anti-α-Gal IgG ELISA	80.8 - 97.9
Human Anti-α-Gal IgM ELISA	88.4 - 93.6
Human Anti-α-Gal IgA ELISA	83.2 - 104.0

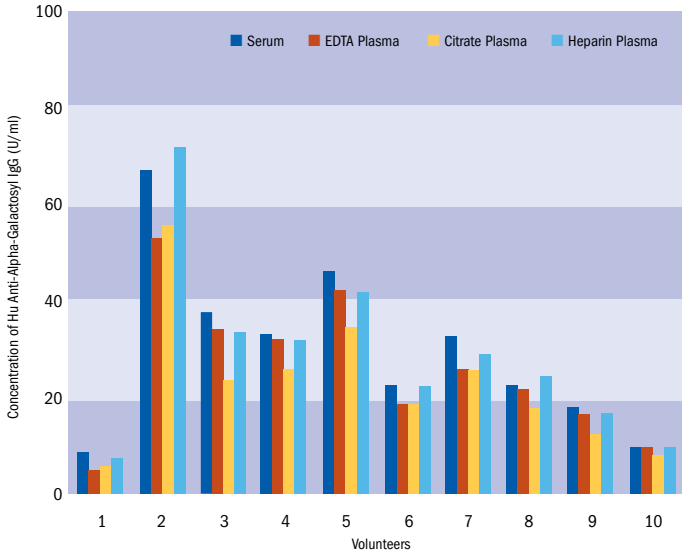
Linearity

Serum samples were serially diluted with Dilution Buffer and assayed. Results are expressed as observed/expected ratio (O/E) range.

ELISA	O/E Range (%)
Human Anti-α-Gal IgG ELISA	83.9 - 96.2
Human Anti-α-Gal IgM ELISA	94.2 - 102.9
Human Anti-α-Gal IgA ELISA	91.5 - 105.7

Effect of sample matrix

EDTA, citrate and heparin plasma samples were compared to respective serum samples from the same 10 individuals. Results obtained for Human Anti-Alpha-Galactosyl IgG ELISA are shown in the graph below for illustration:



The values of coefficient of determination are summarized below:

ELISA	Coefficient of Determination R^2			
	Serum	EDTA plasma	Citrate plasma	Heparin plasma
Human Anti-α-Gal IgG ELISA	-	0.97	0.97	0.98
Human Anti-α-Gal IgM ELISA	-	1.00	0.97	0.99
Human Anti-α-Gal IgA ELISA	-	0.98	0.97	0.99

BioVendor Human Anti-Alpha-Galactosyl IgG ELISA (RD199178100R)

Cross-reactivity

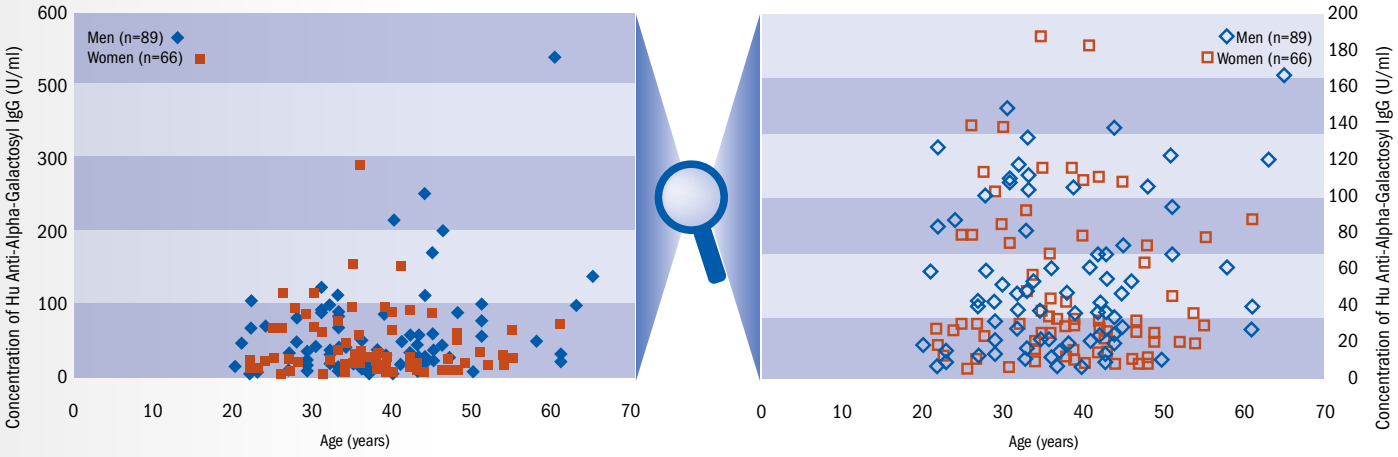
Mammalian serum Sample	Bovine	Cat	Dog	Goat	Hamster	Horse	Monkey	Mouse	Pig	Rabbit	Rat	Sheep
Observed cross-reactivity	no	no	no	no	no	no	yes	no	no	no	no	no

Preliminary Population Data

The following results were obtained when serum samples from 168 unselected donors (91 men + 77 women) 20 - 65 years old were assayed with the BioVendor Human Anti-Alpha-Galactosyl IgG ELISA in our laboratory.

Age and Sex Dependent Distribution of Hu Anti-Alpha-Galactosyl IgG

Sex	Age (years)	n	Mean Anti-α-Gal IgG (U/ml)	Median Anti-α-Gal IgG (U/ml)	SD Anti-α-Gal IgG (U/ml)	Min. Anti-α-Gal IgG (U/ml)	Max. Anti-α-Gal IgG (U/ml)
Male	20-29	18	41.98	34.35	35.13	5.22	126.94
	30-39	30	55.49	46.03	41.32	5.88	148.86
	40-49	33	65.22	32.48	76.40	4.57	305.41
	50-65	10	123.43	80.26	144.55	9.85	534.55
Female	22-29	14	48.45	26.81	42.53	3.06	138.40
	30-39	29	59.85	33.38	69.99	4.00	351.77
	40-49	27	38.75	23.14	42.91	5.30	183.06
	50-61	7	43.30	34.16	25.53	17.98	86.21



A very high degree of variability in concentrations was observed, indicating that the inherent variability of anti-alpha-galactosyl IgG levels appears to be very high in this population.
An appropriate dilution should be assessed by the researcher (due to the high variability of serum anti-alpha-galactosyl IgG level between individuals) in advance to batch measurement. Recommended starting dilution is 100x.

HUMAN ANTI-ALPHA-GALACTOSYL IgG, IgM, IgA

BioVendor Human Anti-Alpha-Galactosyl IgM ELISA (RD199178110R)

Cross-reactivity

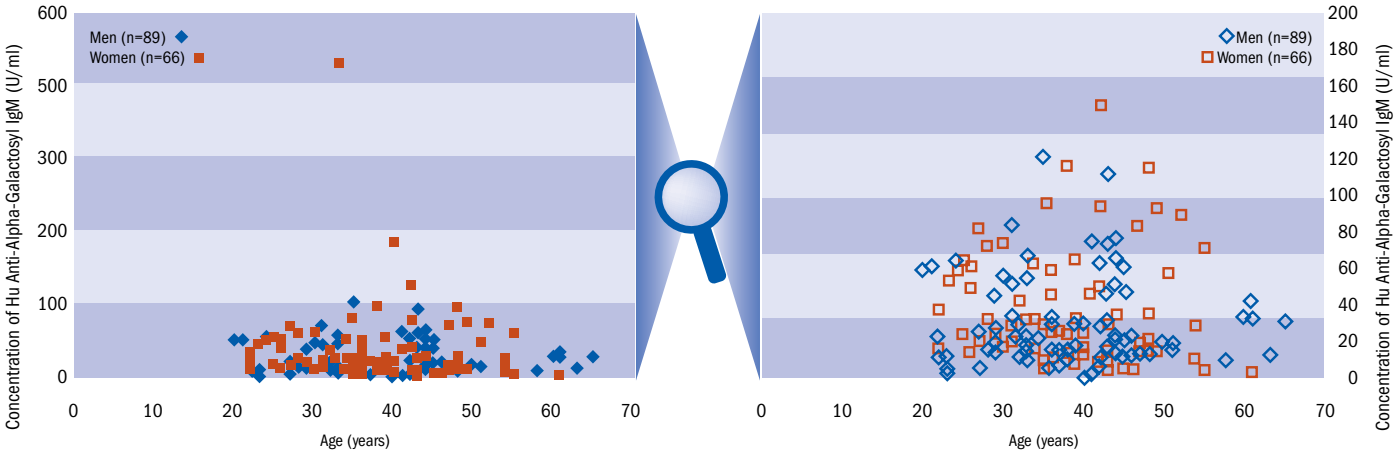
Mammalian serum Sample	Bovine	Cat	Dog	Goat	Hamster	Horse	Monkey	Mouse	Pig	Rabbit	Rat	Sheep
Observed cross-reactivity	no	no	no	no	no	no	yes	no	no	no	no	no

Preliminary Population Data

The following results were obtained when serum samples from 168 unselected donors (91 men + 77 women) 20 - 65 years old were assayed with the BioVendor Human Anti-Alpha-Galactosyl IgM ELISA in our laboratory.

Age and Sex Dependent Distribution of Hu Anti-Alpha-Galactosyl IgM

Sex	Age (years)	n	Mean Anti-α-Gal IgM (U/ml)	Median Anti-α-Gal IgM (U/ml)	SD Anti-α-Gal IgM (U/ml)	Min. Anti-α-Gal IgM (U/ml)	Max. Anti-α-Gal IgM (U/ml)
Male	20-29	18	26.54	21.34	19.27	3.60	65.80
	30-39	30	32.05	24.91	25.03	7.16	123.85
	40-49	33	33.04	24.38	26.85	1.61	113.54
	50-65	10	24.64	20.75	10.02	10.45	42.46
Female	22-29	14	45.14	45.15	21.67	16.00	83.75
	30-39	29	52.02	29.35	92.51	7.70	520.32
	40-49	27	44.19	19.82	51.02	5.53	222.03
	50-61	7	39.11	29.95	32.55	3.86	91.16



A very high degree of variability in concentrations was observed, indicating that the inherent variability of anti-alpha-galactosyl IgM levels appears to be very high in this population.
An appropriate dilution should be assessed by the researcher (due to the high variability of serum anti-alpha-galactosyl IgM level between individuals) in advance to batch measurement. Recommended starting dilution is 100x.

QUANTITATIVE DETERMINATION OF HUMAN ANTI-ALPHA-GALACTOSYL IgG, IgM, IgA

BioVendor Human Anti-Alpha-Galactosyl IgA ELISA (RD199178120R)

Cross-reactivity

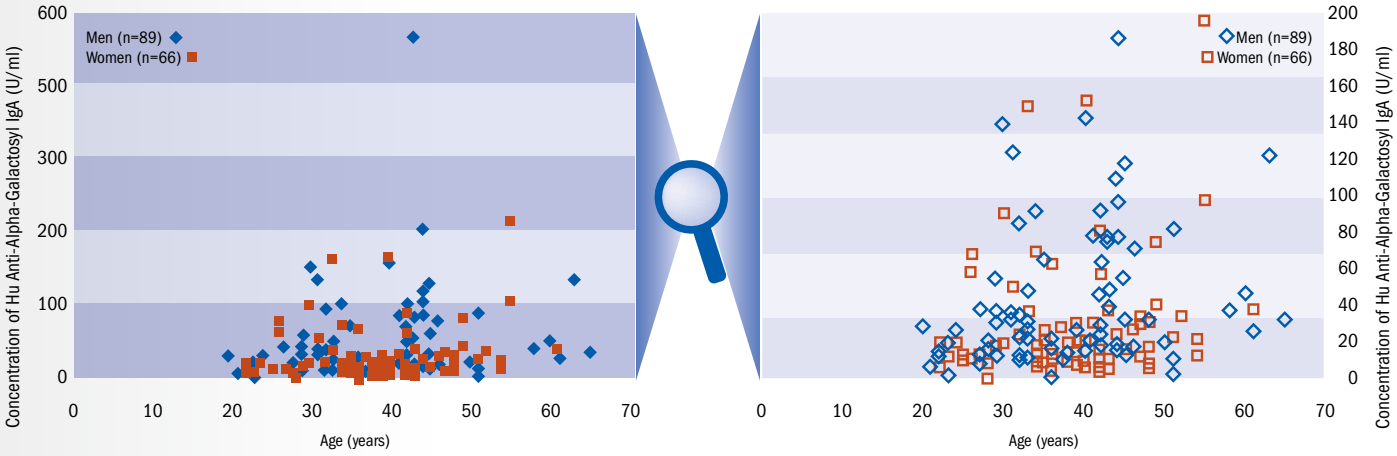
Mammalian serum Sample	Bovine	Cat	Dog	Goat	Hamster	Horse	Monkey	Mouse	Pig	Rabbit	Rat	Sheep
Observed cross-reactivity	no	yes	yes	no	no	yes	yes	no	no	no	no	no

Preliminary Population Data

The following results were obtained when serum samples from 168 unselected donors (91 men + 77 women) 20 - 65 years old were assayed with the BioVendor Human Anti-Alpha-Galactosyl IgG ELISA in our laboratory.

Age and Sex Dependent Distribution of Hu Anti-Alpha-Galactosyl IgA

Sex	Age (years)	n	Mean Anti-α-Gal IgA (U/ml)	Median Anti-α-Gal IgA (U/ml)	SD Anti-α-Gal IgA (U/ml)	Min. Anti-α-Gal IgA (U/ml)	Max. Anti-α-Gal IgA (U/ml)
Male	20-29	18	20.00	15.01	13.17	2.09	54.69
	30-39	30	33.80	22.39	33.42	0.00	138.59
	40-49	33	63.79	39.78	76.15	6.16	423.00
	50-65	10	40.11	29.06	34.24	2.27	121.73
Female	22-29	14	18.90	12.09	19.02	0.16	67.90
	30-39	29	29.16	19.36	30.64	2.79	148.40
	40-49	27	28.48	16.48	31.17	4.27	151.37
	50-61	7	60.00	33.91	60.94	12.22	195.19



A very high degree of variability in concentrations was observed, indicating that the inherent variability of anti-alpha-galactosyl IgA levels appears to be very high in this population.
An appropriate dilution should be assessed by the researcher (due to the high variability of serum anti-alpha-galactosyl IgA level between individuals) in advance to batch measurement. Recommended starting dilution is 100x.

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