

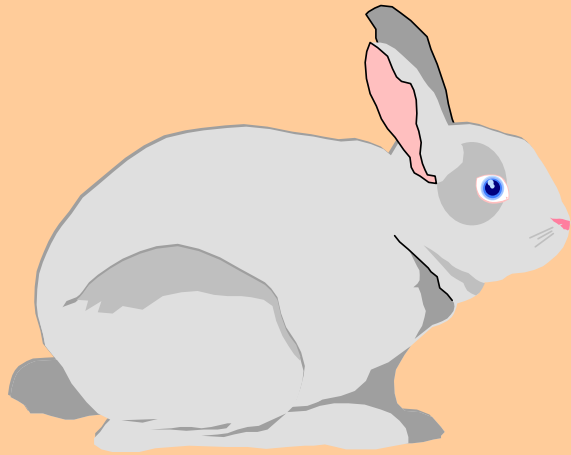
Anti-galactosyl Natural Antibodies in the Patients with Antibody Deficiency

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Natural Antibodies

- Natural Antibodies against carbohydrate antigens are thought to be produced through life as a polyclonal response to antigenic stimulation by gastrointestinal bacteria but their origin is uncertain.



Natural Antibodies:

„Classical investigation“ - rabbit

Xenoagglutinins were determined by direct **HEMAGGLUTINATION** test with **rabbit erythrocytes**.

New possibility:

$Gal\alpha 1-3Gal$

was identified as the epitope of rabbit erythrocytes.

Characterization of Natural Antibodies

Natural antibodies against carbohydrate antigens:

- Xenoreactive **IgM** Ab = 1,8 - 4,1%, resp. 3,9 - 8,0%
of total IgM
- Xenoreactive **IgG** Ab = 1 - 2,4% of total IgG

McMorrow, I. M., Comrack, C. A., Sachs, D. H., DerSimonian, H. (1997). Heterogeneity of human anti-pig natural antibodies cross-reactive with the Gal(α 1,3)Galactose epitope. *Transplantation* 64: 501-10.

Development of the method - NA

ELISA with antigen **Gal α 1-3Gal**
in the isotypes **IgM, IgG** and **IgA**,
enable to detect
„classical natural antibodies against rabbit
erythrocytes“.

Rieben, R., von Allmen, E., Korchagina, E. Y., Nydegger, U. E., Neethling, F. A., Kujundzic, M., Koren, E., Bovin, N. V., Cooper, D. K. C. (1995). Detection, immunoabsorption, and inhibition of cytotoxic activity of anti- α Gal antibodies using newly developed substances with synthetic Gal α 1-3Gal disaccharide epitopes. *Xenotransplantation* 2: 98-106.

Development and determination of

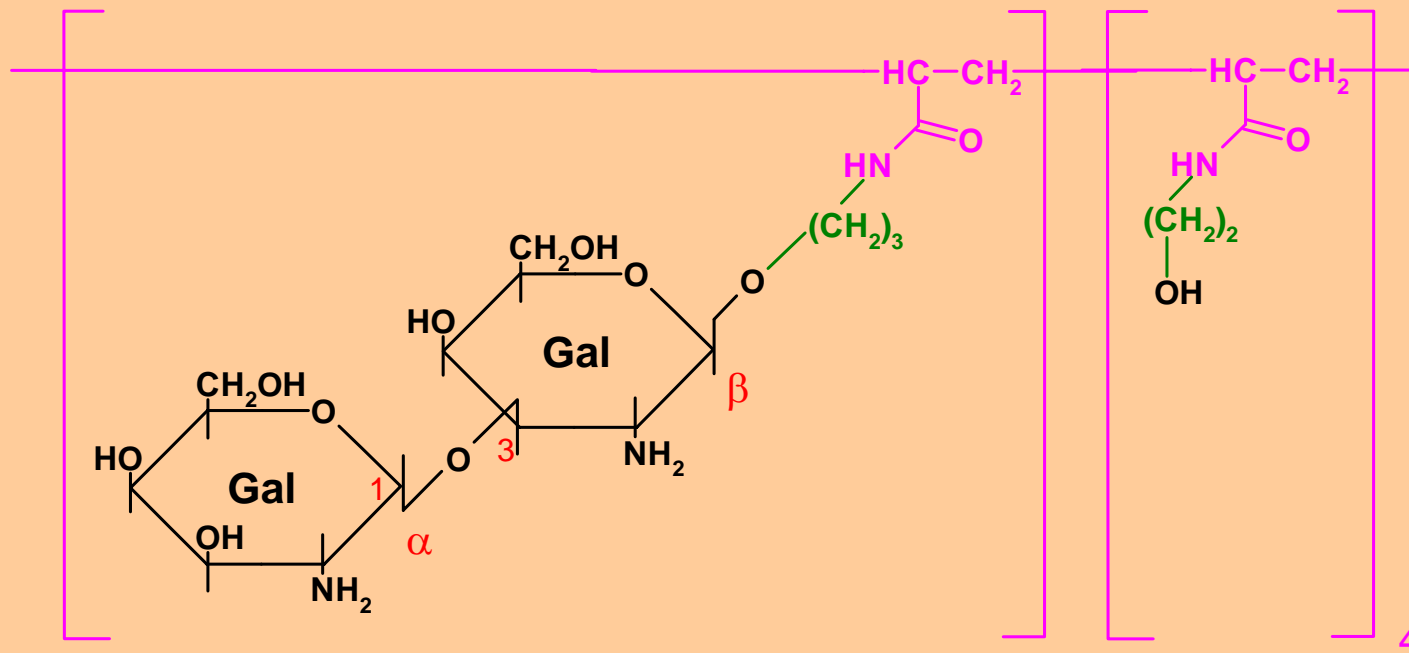
**IgM, IgG and IgA anti- α -galactosyl
natural antibodies (NA)**

by ELISA

and comparison with hemagglutination.

ELISA for Natural antibodies

- antigen:
PAA - β -disaccharide Gal α 1-3Gal



(PAA-B_{di} Lectinity Holdings, Inc., Moscow, Russia)

ELISA

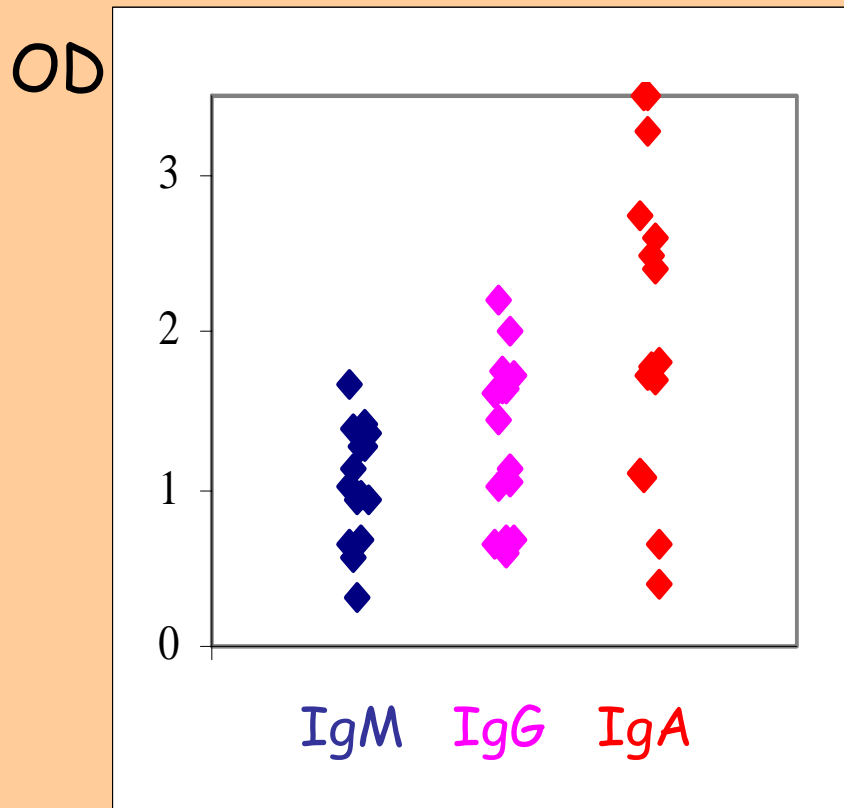
for

IgM-, IgG- and IgA-NA

- healthy donors
 - COVID
- neonates

Occurrence of anti- α Gal NA in healthy donors

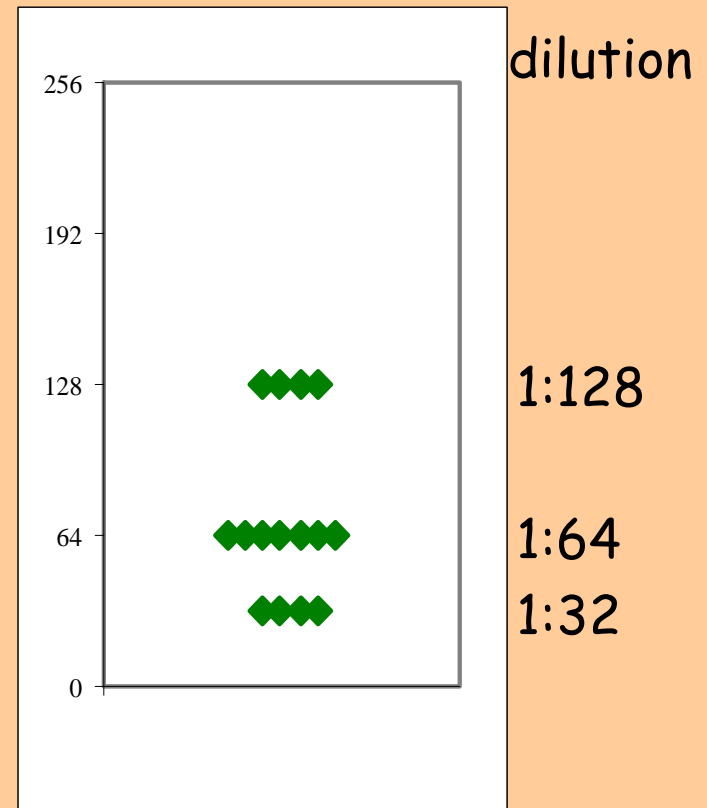
ELISA



n = 15

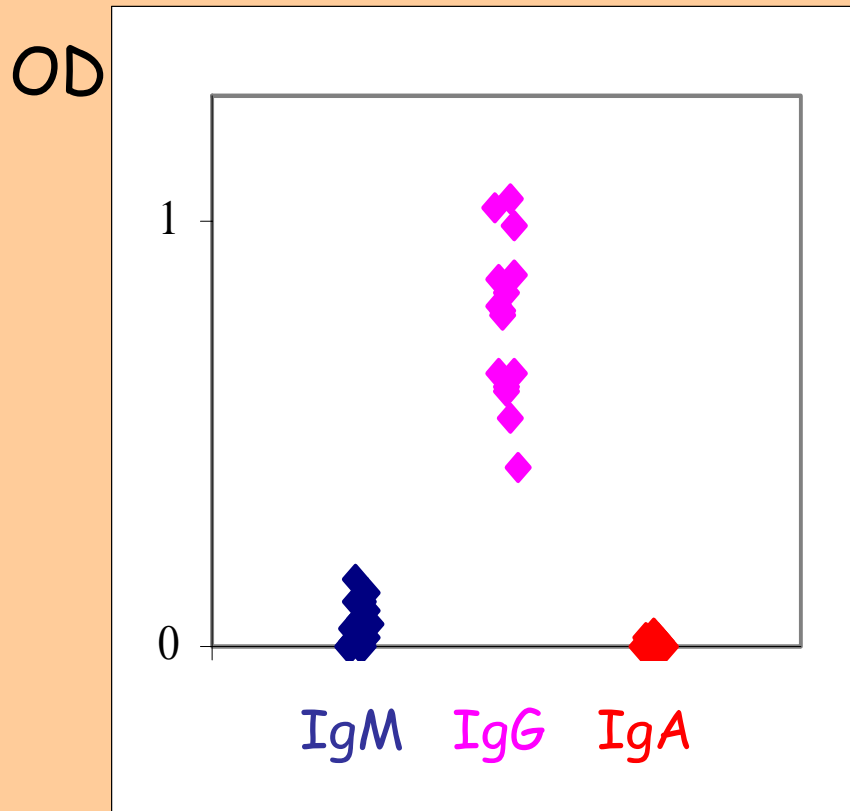
dil. 1:40

AGGLUTINATION



Occurrence of anti- α Gal NA in CVID

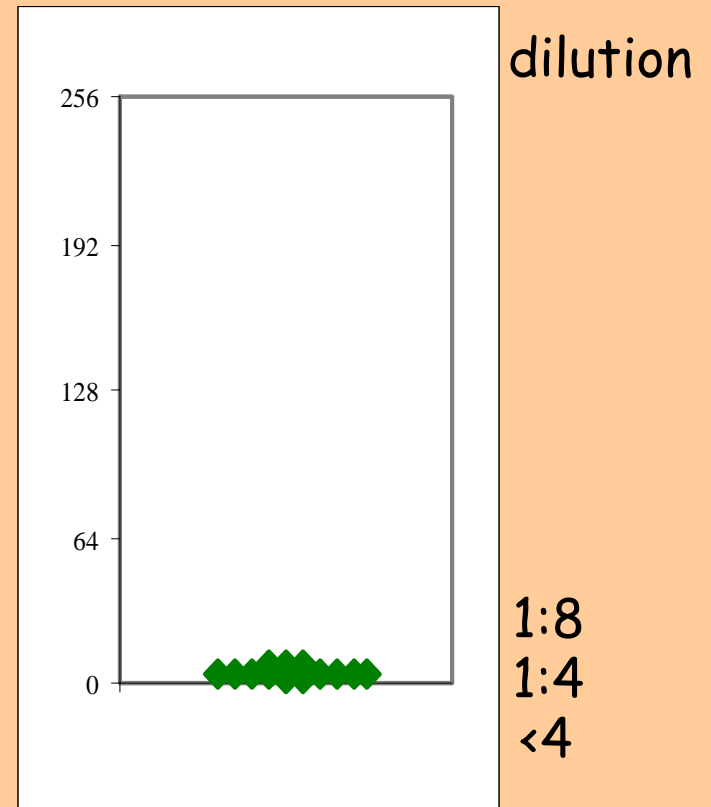
ELISA



n = 15

dil. 1:40

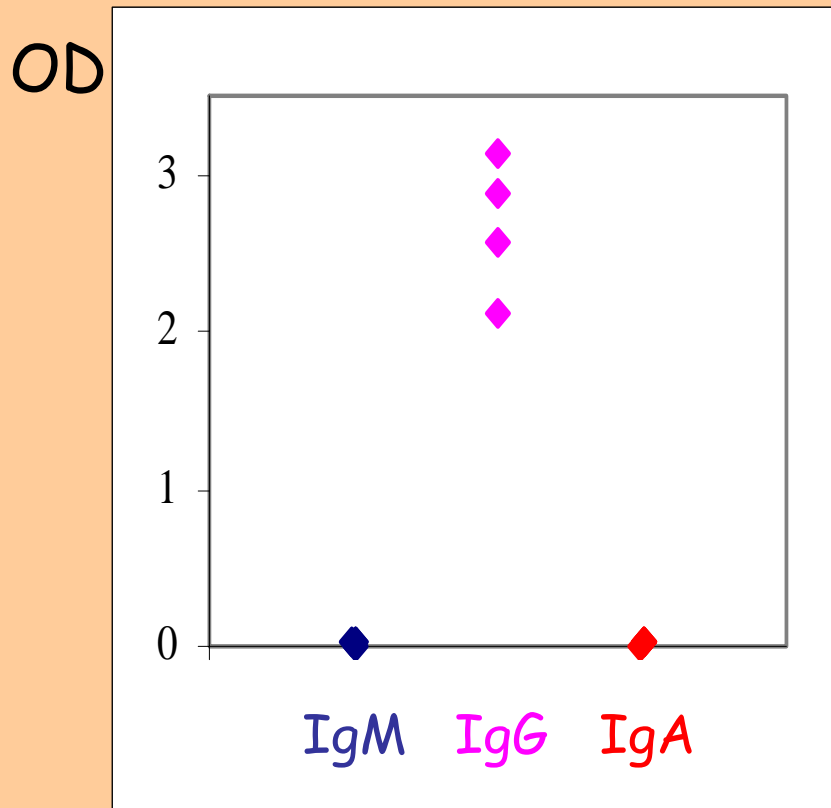
AGGLUTINATION



1:8
1:4
<4

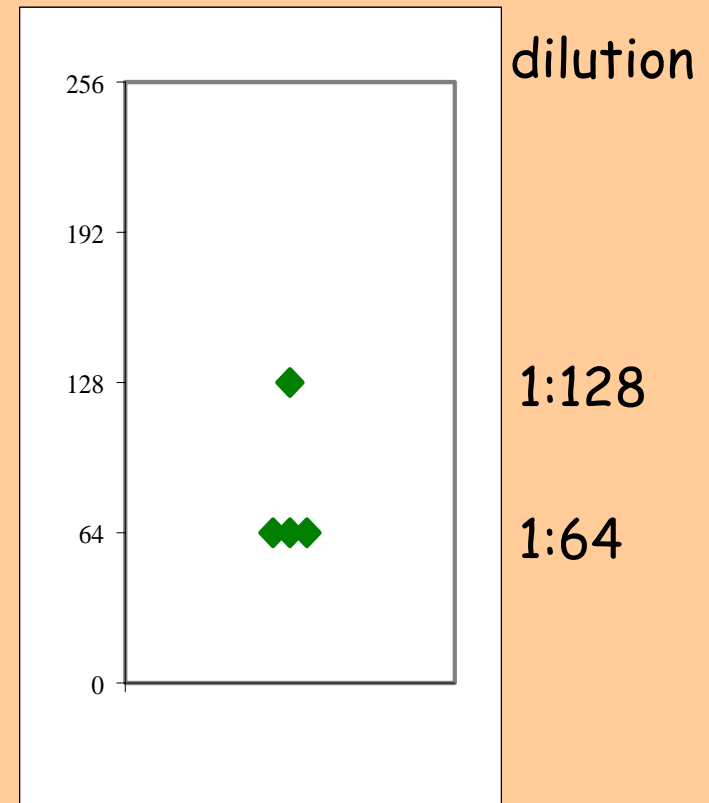
Occurrence of anti- α Gal NA in IVIG

ELISA



Endobulin n = 4 dil. 1:40

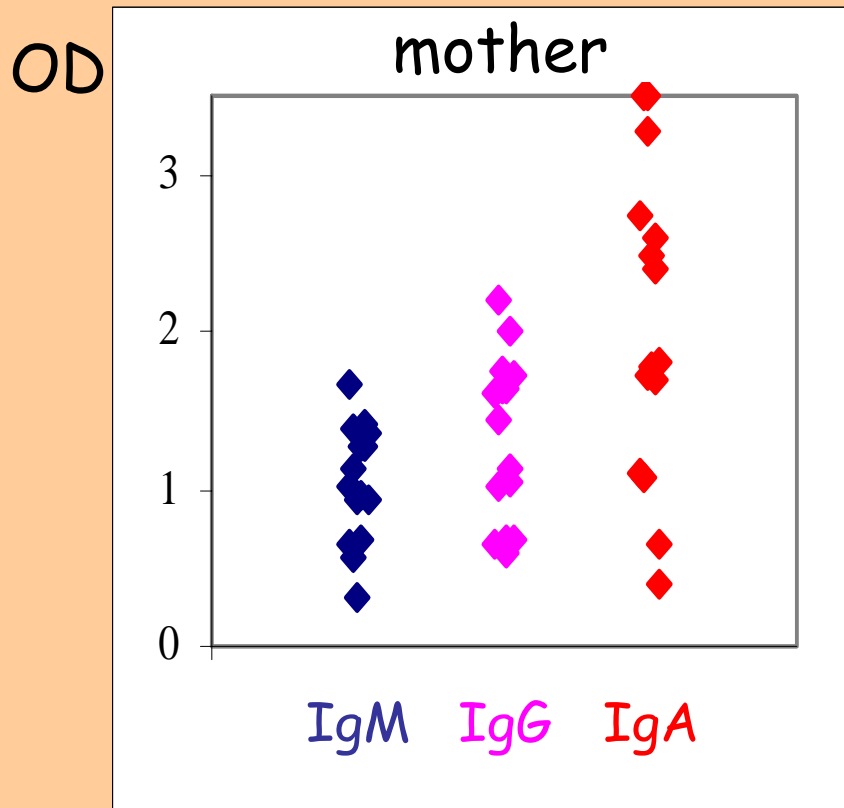
AGGLUTINATION



dilution
1:128
1:64

Occurrence of anti- α Gal NA in cord blood

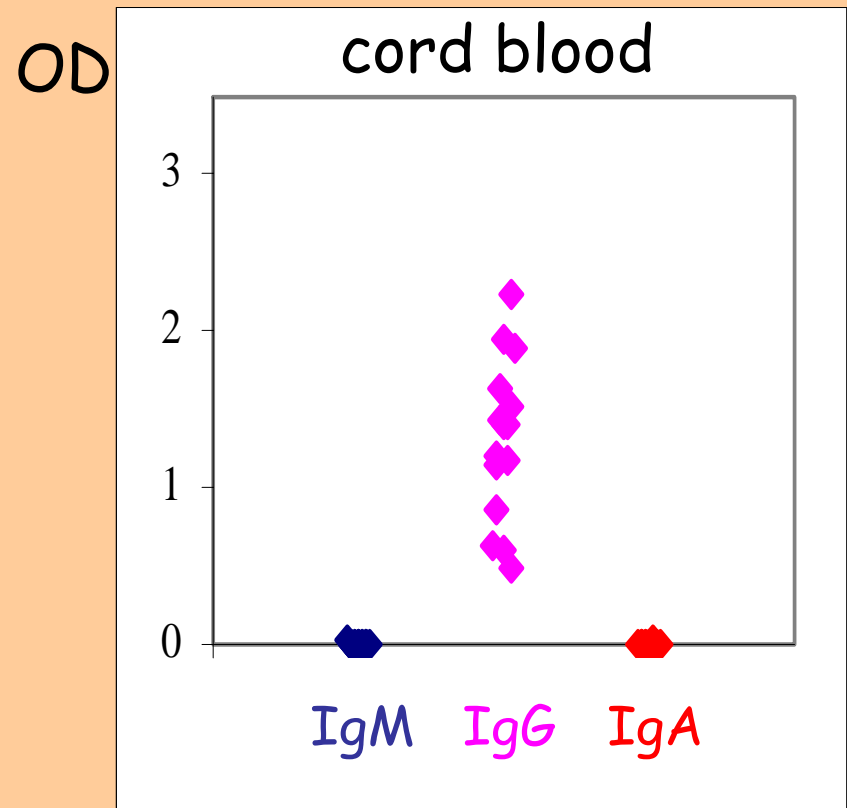
ELISA



n = 15

dil. 1:40

ELISA



n = 15

dil. 1:40

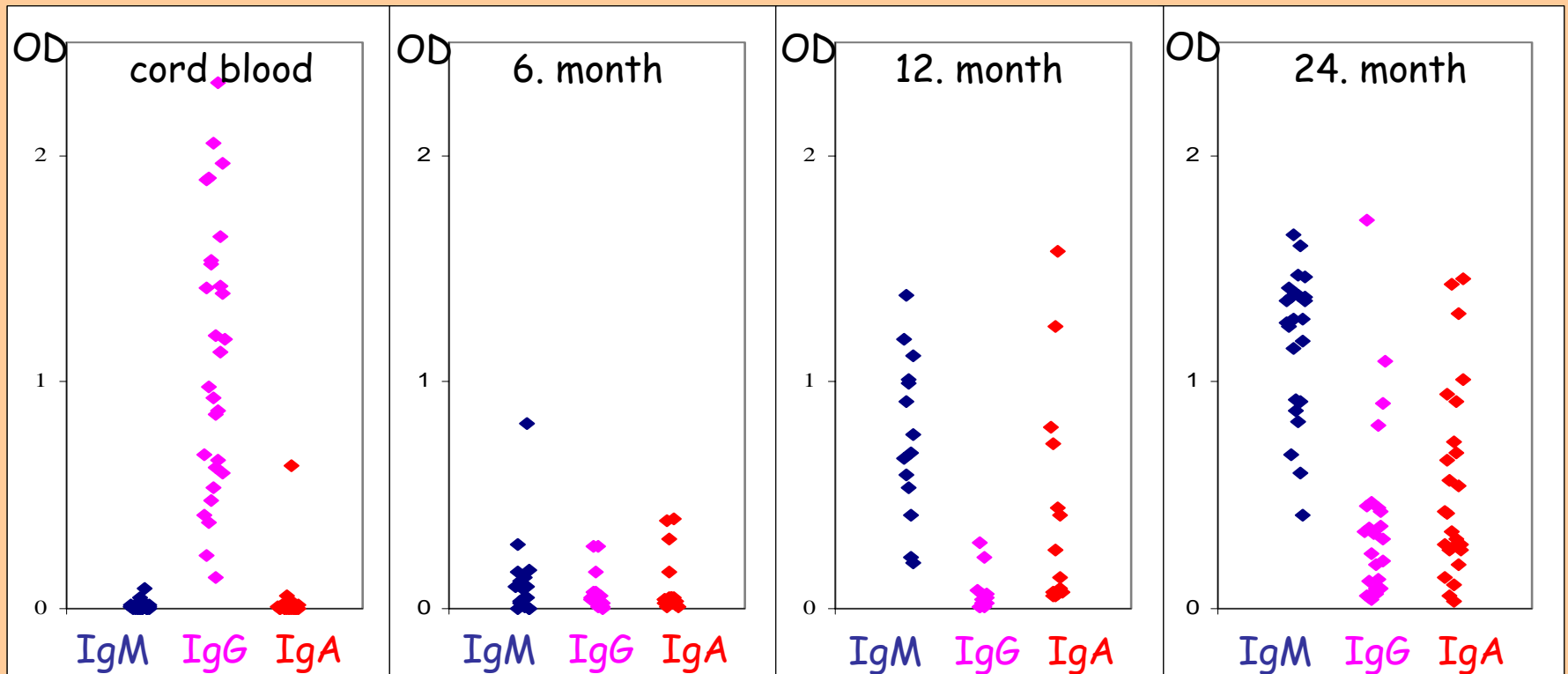
Occurrence of anti- α Gal NA in neonates, followed in time

ELISA

ELISA

ELISA

ELISA



n = 28

n = 19

n = 14

n = 25

dil. 1:40

dil. 1:40

dil. 1:40

dil. 1:40

With our specific ELISA using $\text{Gal}\alpha 1-3\text{Gal}$ antigen we determine natural antibodies in different Ig class such as IgM, IgG and IgA and so specific investigate up to 95% of xenoreactive natural antibodies.

With the new reproducible assay we are able to detect natural antibodies in patients with CVID, particularly in low concentrations in IgM and IgA isotypes.

Investigation of genetically conserved natural antibodies may permit functional evaluation and characterization of the differentiation state of B lymphocytes.

As a perspective $\text{Gal}\alpha 1-3\text{Gal}$ antigen could also be appropriate for the detection of natural antibodies at the cellular level in ELISPOT assay.