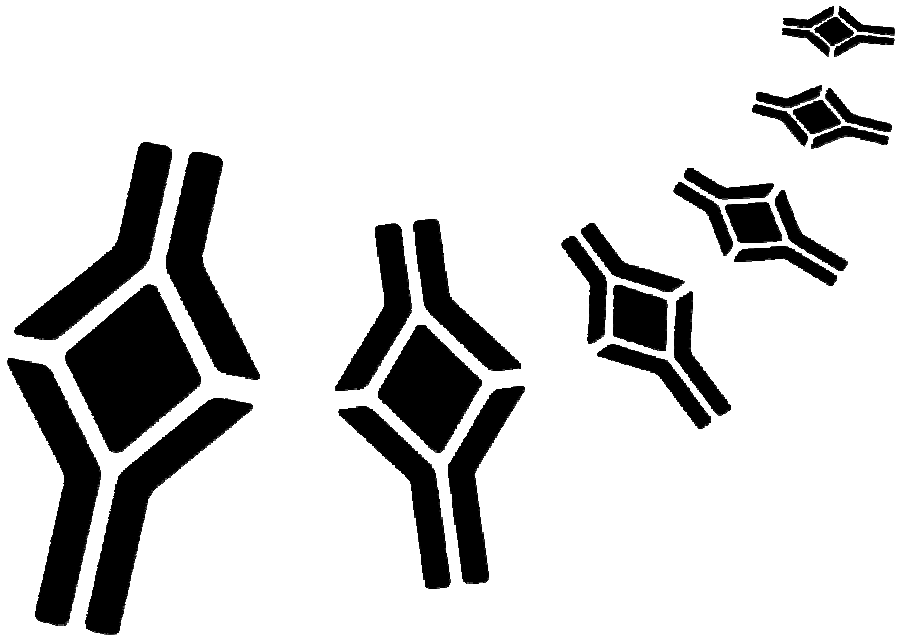
BioVendor
Research
and Diagnostic Products



HUMAN ANTI-IGA ISOTYPE IGG ELISA

Product Data Sheet

Cat No.: RD199145100R

For Research Use Only

VERSION 42 100311 44

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD199145100R Human Anti-IgA isotype IgG ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human anti-IgA antibodies in the IgG class.

» Features

- **It is intended for research use only**
- The total assay time is less than 3 hours
- The kit measures IgG anti-IgA antibodies in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Calibrator is human serum based
- Quality Controls are human serum based. No animal sera are used
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2 - 8° C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Anaphylactoid reaction induced by IgG anti-IgA antibodies may occur in individuals with IgA deficiency. The incidence of IgA deficiency is quite common in the population, with its frequency reaching nearly 1:400. Formation of immunocomplexes of antibodies (IgG anti-IgA) with antigen (IgA) leads to complement activation. Anaphylactoid reaction that release vasoactive anaphylatoxins (C3a, C4a, C5a) is a serious complication in transfusions or immunoglobuline replacement therapy. Like anaphylactic reaction, it is accompanied by hives, hypotension, dispnoea with bronchospasm or stridor.
Anti-IgA antibodies are found in patients with undetectable IgA levels. In about 10% of patients with IgA deficiency (IgAD) and with common variable immunodeficiency (CVID), anti-IgA antibodies are present. A causal relationship has been between IgG anti-IgA antibodies and the development of anaphylactoid reactions has been demonstrated, especially for cases when anti-IgA antibodies are in higher concentrations.
Patients with IgA deficiency who need a blood transfusion or blood derivatives substitution should receive negative IgA blood components or an autotransfusion should be performed. Measurement of anti-IgA antibodies should be carried out in patients requiring this treatment.
The recommendation for subjects with known anaphylactoid reaction is to perform the test for anti-IgA at the interval of at least ten days after the response (the onset of antibodies production). Alternative subcutaneous immunoglobulin replacement therapy (SCIG) may be considered if anaphylactoid reactions to intravenous gammaglobulin therapy (IVIG) have occured and if anti-IgA antibodies are present.

<u>Areas of investigation:</u>
Hematology and blood transfusion, blood derivatives, replacement therapy
Immunology – immunodeficiency and substitution therapy

4. TEST PRINCIPLE

Human polyclonal immunoglobulins A (IgA) are bound to the microplate wells. Calibrators, control sera and samples of sera are pipetted into the wells and incubated. Anti-IgA antibodies present in the samples are bound to immobilized IgA molecules. The unbound substances are removed by the washing step. The specific polyclonal antibody against human immunoglobulin G (IgG) is then added into the wells. This antibody is conjugated with horseradish peroxidase (HRP) and it is linked to the immobilized complex IgA – IgG anti-IgA during incubation. The unbound conjugate is removed during the subsequent washing step, and then the substrate solution tetramethylbenzidine (TMB) is added into the wells. The enzyme reaction causes the solution in the wells to turn color (blue) and the intensity of the color is directly proportional to the amount of linked IgG anti-IgA antibodies. Development of the color is stopped by an acid stop reagent, and the absorbance of the resulting yellow product is measured. The concentration of IgG anti-IgA antibodies in unknown samples is determined from the calibration curve, which is constructed by plotting absorbance values of calibrators against their known concentration.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of human serum origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. These materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 ml
Master Calibrator	ready to use	1.4 ml
Quality Control POSITIVE	ready to use	0.7 ml
Quality Control NEGATIVE	ready to use	0.7 ml
Inhibitory Control POSITIVE	lyophilized	1 vial
Inhibitory Control NEGATIVE	lyophilized	1 vial
Dilution Buffer	ready to use	50 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5 – 1 000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Thermostatic box adjustable to 37° C
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550 – 650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

» **All reagents need to be brought to room temperature prior to use**

» **Always prepare only the appropriate quantity of reagents for your test**

» **Do not use components after the expiration date marked on their label**

- **Assay reagents supplied ready to use:**

Antibody Coated Microtiter Strips
Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2 - 8° C and protected from the moisture.

Conjugate Solution
Dilution Buffer
Substrate Solution
Stop Solution
Stability and storage:
Opened reagents are stable 3 months when stored at 2 - 8° C.

Human IgG anti-IgA antibody Master Calibrator
Prepared Master Calibrator is ready to use, do not dilute it.
Mix well before use (not to foam). Vortex is recommended.
The resulting concentration in the stock solution is **100 U/ml**.

Prepare set of calibrators using Dilution Buffer as follows:

Volume of Calibrator	*Dilution Buffer*	*Concentration*
Stock	-	100 U/ml
250 µl of stock	250 µl	50 U/ml
250 µl of 50 U/ml	250 µl	25 U/ml
250 µl of 25 U/ml	250 µl	12.5 U/ml
250 µl of 12.5 U/ml	250 µl	6.25 U/ml
250 µl of 6.25 U/ml	250 µl	3.13 U/ml
250 µl of 3.13 U/ml	250 µl	1.56 U/ml

Prepared set of calibrators are ready to use, do not dilute them.

Stability and storage:
Opened Master Calibrator is stable 3 months when stored at 2 - 8° C.
Do not store the diluted Calibrator solutions.

Quality Controls POSITIVE, NEGATIVE
Prepared Quality Controls (QC POSITIVE, QC NEGATIVE) are ready to use, do not dilute them.
Refer to the Certificate of Analysis for current Quality Control concentration!!!
Mix well before use (not to foam). Vortex is recommended.
Stability and storage:
Opened Quality Controls are stable 3 months when stored at 2 - 8° C.

- **Assay reagents supplied concentrated:**

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten fold in distilled (deionized) water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.
Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2 - 8° C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2 - 8° C.

10. PREPARATION OF SAMPLES

The kit measures IgG anti-IgA antibodies in serum and plasma (EDTA, citrate, heparin).

Samples can be assayed immediately after collection, after storage for a week at 2 - 8° C, or after long-term storage at -20° C (-70° C). Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 100x with Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 495 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Serum and plasma samples should be stored at -20° C or preferably at -70° C or lower for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at 2 - 8° C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of IgG anti-IgA.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Calibrators, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at 37° C for **1 hour**, no shaking.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25° C) for **1 hour**, no shaking.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Solution in wells will turn blue. Do not shake the plate during the incubation.
9. Stop the colour development by adding **100 µl** of Stop Solution. Solution will turn yellow.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 – 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 9.**

Note: If some samples and calibrators have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new calibration curve, constructed using the values measured at 405 nm, is used to determine IgG anti-IgA antibody concentration of off-scale calibrators and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Calibrator 100**	**QC POSIT.**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Calibrator 50**	**QC NEGAT.**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Calibrator 25**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Calibrator 12.5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Calibrator 6.25**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Calibrator 3.13**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Calibrator 1.56**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the mean absorbance (Y) of Calibrators against the known concentration (X) of Calibrators in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of IgG anti-IgA antibodies U/ml in samples.

Alternatively, the *logit log* function can be used to linearize the calibration curve, i.e. *logit* of the mean absorbance (Y) is plotted against *log* of the known concentration (X) of Calibrators.

Concentration obtained from calibration curve is the final concentration of IgG anti-IgA antibodies in the sample. Dilution factor (100x) is calculated. The results are not to be multiplied by dilution factor.
Dilution factor for samples with dilution higher than the basic dilution (100x) in ELISA is shown in the Chapter 13 in the paragraph Limit of Assay.

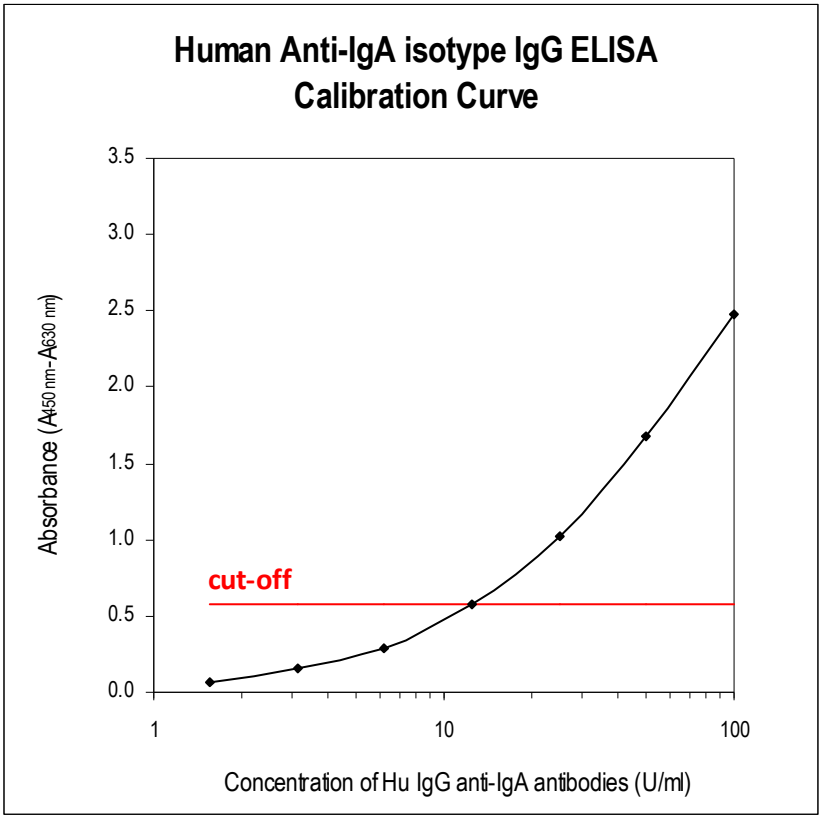


Figure 2: Typical Calibration Curve for Human Anti-IgA isotype IgG ELISA.

- **Cut-off limit**

Cut-off limit that is at **4th point of the calibration curve** in our ELISA test represents the limit of clinical relevance of measured levels of IgG anti-IgA. It was determined on the basis of existing studies and clinical experience with the level of IgG anti-IgA antibodies.

The measured levels of IgG anti-IgA antibodies less than the cut-off value have no clinical relevance and we indicate them as **negative**.

The measured levels of IgG anti-IgA antibodies greater than or equal to the cut-off value have clinical relevance and we indicate them as **positive**.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Anti-IgA isotype IgG ELISA are presented in this chapter

- **Sensitivity**

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3xSD_{blank}$) is calculated from the real IgG anti-IgA antibody values in wells and is 0.71 U/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of Assay**

Results exceeding IgG anti-IgA antibody level of 100 U/ml should be repeated with more diluted samples. **When diluting more than 100 times, it has to be taken into account that the results must be multiplied by the dilution factor divided by 100.** For example, when the samples are diluted 200 times, the results must be multiplied by 200/100 (= 2).

- **Specificity**

The method measures human IgG anti-IgA antibodies. The detection antibodies used in this kit are specific toward human IgG antibodies. To avoid false-positive results, immunoreagencies for eventual execution of so-called inhibition test are supplied with the kit.

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

There was no cross-reactivity observed with any of the mentioned mammalian species above when using the recommended sample dilution 100x. However, non-specific signal was measured in pig and monkey sera at dilutions less than the basic recommended dilution (17x).

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (U/ml)*	*SD (U/ml)*	*CV (%)*
1	69.67	2.23	3.2
2	34.08	1.83	5.4
3	28.24	1.62	5.8

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

Sample	*Mean (U/ml)*	*SD (U/ml)*	*CV (%)*
1	63.98	3.01	4.7
2	31.13	1.30	4.2
3	15.56	1.11	7.1

- **Spiking Recovery***

Defined amounts of human anti-IgA antibodies of IgG class were added to serum samples positive for IgG anti-IgA antibodies, and then their total concentration was determined.

Sample	***O**bserved (U/ml)*	***E**xpected (U/ml)*	*Recovery **O/E** (%)*
1	15.55	-	-
	21.26	21.49	99
	22.81	27.43	83
	39.84	39.30	101
2	11.87	-	-
	17.37	15.81	110
	22.11	19.75	112
	28.96	27.62	105

**results are not multiplied by dilution factor*

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (U/ml)*	***E**xpected (U/ml)*	*Recovery **O/E** (%)*
1	-	30.08	-	-
	2x	12.92	15.04	86
	4x	6.10	7.52	81
	8x	3.38	3.76	90
2	-	236.06	-	-
	2x	115.32	118.03	98
	4x	55.42	59.02	94
	8x	28.91	29.51	98

- **Effect of sample matrix**

EDTA, citrate and heparin plasma samples were compared to respective serum samples from the same 7 individuals. Results are shown below:

Volunteer No.	*Serum (U/ml)*	*Plasma (U/ml)*		
		EDTA	*Citrate*	*Heparin*
1	80.34	77.81	55.34	68.91
2	13.33	16.15	15.09	14.08
3	278.10	204.81	194.62	156.88
4	86.12	60.85	56.09	58.27
5	16.74	15.63	13.07	15.76
6	10.90	10.05	9.29	9.58
7	209.65	218.70	215.24	259.49
Mean (U/ml)	**99.31**	**86.29**	**79.82**	**83.28**
Mean Plasma/Serum (%)	**-**	**86.9**	**80.4**	**83.9**
Coefficient of determination R^2	**-**	**0.94**	**0.93**	**0.76**

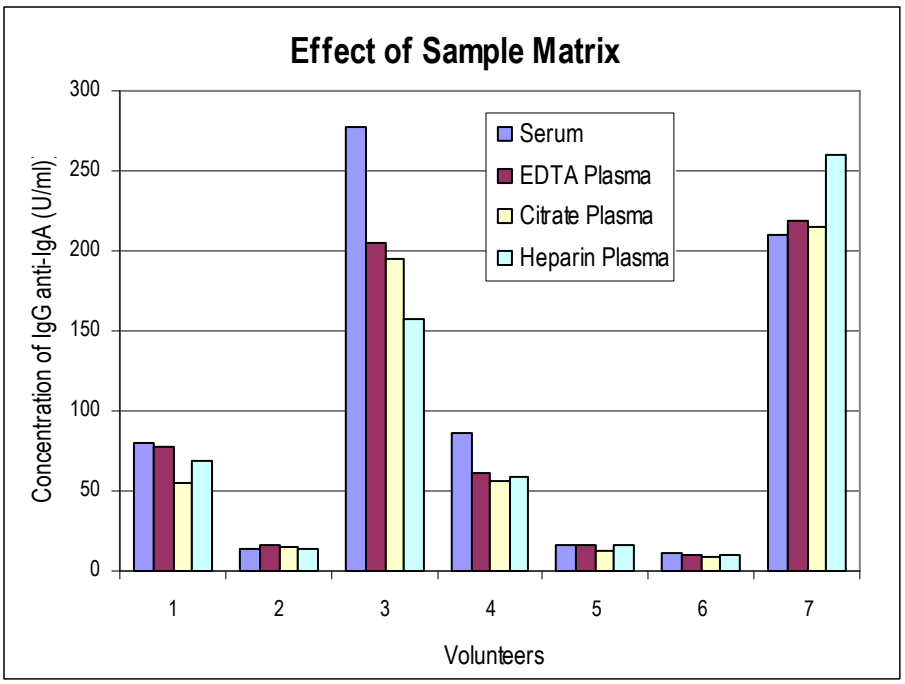


Figure 3: IgG anti-IgA antibody levels measured using Human Anti-IgA isotype IgG ELISA from 7 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2 - 8° C**

No significant decrease was detected in the concentration of human IgG anti-IgA antibodies after 28 days of storage at 2 - 8° C. To avoid microbial contamination, samples were treated with thimerosal, resulting in the final concentration of 0.01%. Samples should be stored at -20° C or preferably at -70° C or lower for long-term storage.

Sample	*Incubation Temp, Period*	*Serum (U/ml)*	*Plasma (U/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-80° C	94.74	76.97	71.75	91.75
	2 - 8° C, 14	103.04	77.05	70.81	84.79
	2 - 8° C, 28	78.02	81.26	57.13	74.19
2	-80° C	193.98	205.00	191.73	159.12
	2 - 8° C, 14	229.13	179.78	200.08	147.58
	2 - 8° C, 28	216.78	168.24	235.77	155.46
3	-80° C	12.46	14.43	9.57	12.27
	2 - 8° C, 14	14.30	11.60	10.70	11.79
	2 - 8° C, 28	10.62	12.92	9.06	11.10

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human IgG anti-IgA antibodies in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	*Number of f/t cycles*	*Serum (U/ml)*	*Plasma (U/ml)*		
			EDTA	*Citrate*	*Heparin*
1	1x	91.61	83.52	70.97	94.87
	3x	106.74	87.17	74.30	94.27
	5x	87.12	81.74	69.00	93.53
2	1x	209.74	195.91	208.63	163.22
	3x	236.46	192.94	207.66	163.36
	5x	247.14	204.64	218.41	181.12
3	1x	11.67	10.14	9.74	12.10
	3x	13.13	10.92	9.60	11.47
	5x	11.04	10.50	9.31	12.33

- **Reference range**

It is recommended that each laboratory include its own panel of positive control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for IgG anti-IgA antibody levels with the assay.

- **Normal values**

Levels of IgG anti-IgA antibodies are negative in sera of healthy donors.

14. DEFINITION OF THE CALIBRATOR

The Calibrator used in this kit is IgG anti-IgA antibody of human serum origin.

15. INHIBITION TEST

False positive results can be excluded and specificity confirmed by performing an antibody neutralization test (inhibition test). The inhibition test is carried out with samples for which the measured level of IgG anti-IgA antibodies in the ELISA test was higher or equal to the cut-off limit (i.e. positive samples).

THE TEST PRINCIPLE

Two inhibitory controls (positive and negative) are supplied with the kit for the needs of the inhibition test. The positive control contains serum IgA, whereas the negative control does not. If these controls are added to the samples that initially tested positive, a true positive result (false-positive excluded) is confirmed when (1) neutralization occurs with the addition of the positive inhibitory control and (2) no neutralization occurs with the addition of negative inhibitory control. In the case of a positive inhibition test (i.e. true positive result), the subsequently measured amount of IgG anti-IgA antibodies in the sample will be quantitatively reduced by the positive inhibitory control while the originally measured amount remains unchanged with the addition of negative inhibitory control.

PREPARATION OF REAGENTS

Assay reagents supplied lyophilized:

Inhibitory Controls (POSITIVE, NEGATIVE)

Reconstitute each Inhibitory Control (POSITIVE and NEGATIVE) with **100 µl** of Dilution Buffer just prior to the assay. Let it dissolve at least 30 minutes with occasional gentle shaking (not to foam). **Mix well** before use (not to foam). Vortex is recommended.

Stability and storage:

Reconstituted Inhibitory Controls are stable 3 months when stored at 2 - 8° C.

PROCEDURE FOR CARRYING OUT THE INHIBITION TEST

Positive inhibitory control:

1. Pipet **85 µl** of Dilution Buffer into the empty test tube.
2. Add **5 µl** of reconstituted Inhibitory Control POSITIVE into test tube.
3. Add **10 µl** of sample, in which you want to exclude false-positive results.
4. **Mix well** (not to foam). Vortex is recommended.
5. Incubate the test tube at room temperature (ca. 25° C) for **30 minutes**.
6. Add **900 µl** of Dilution Buffer (the final dilution of sample is now 100x).
7. **Mix well** (not to foam). Vortex is recommended.
8. Now this tested sample is prepared for measure of IgG anti-IgA level in the ELISA test.
9. Pipet **100 µl** of prepared sample from inhibition test into well/s next to **100 µl** diluted Calibrators, Quality Controls, Dilution Buffer (= Blank) and diluted samples (duplicates are recommended) into the appropriate wells of the microtiter plate.
10. Following step 2 (see Chapter 11).

Negative control of inhibition:

1. Pipet **85 µl** of Dilution Buffer into the empty test tube.
2. Add **5 µl** of reconstituted Inhibitory Control NEGATIVE into test tube.
3. Add **10 µl** of sample, in which you want to exclude false-positive results.
4. **Mix well** (not to foam). Vortex is recommended.
5. Incubate the test tube at room temperature (ca. 25° C) for **30 minutes**.
6. Add **900 µl** of Dilution Buffer (the final dilution of sample is now 100x).
7. **Mix well** (not to foam). Vortex is recommended.
8. Now this tested sample is prepared for measure of IgG anti-IgA level in the ELISA test.
9. Pipet **100 µl** of prepared sample from inhibition test into well/s next to **100 µl** diluted Calibrators, Quality Controls, Dilution Buffer (= Blank) and diluted samples (duplicates are recommended) into the appropriate wells of the microtiter plate.
10. Following step 2 (see Chapter 11).

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Calibrator 100**	Sample 1	Sample 1 (with Inh.Con.POS.)	Sample 1 (with Inh.Con.NEG.)	Sample 11	Sample 19
B	**Calibrator 50**	Sample 2	Sample 2 (with Inh.Con.POS.)	Sample 2 (with Inh.Con.NEG.)	Sample 12	Sample 20
C	**Calibrator 25**	Sample 3	Sample 3 (with Inh.Con.POS.)	Sample 3 (with Inh.Con.NEG.)	Sample 13	Sample 21
D	**Calibrator 12.5**	Sample 4	Sample 4 (with Inh.Con.POS.)	Sample 4 (with Inh.Con.NEG.)	Sample 14	Sample 22
E	**Calibrator 6.25**	Sample 5	Sample 5 (with Inh.Con.POS.)	Sample 5 (with Inh.Con.NEG.)	Sample 15	Sample 23
F	**Calibrator 3.13**	Sample 6	Sample 6 (with Inh.Con.POS.)	Sample 6 (with Inh.Con.NEG.)	Sample 16	Sample 24
G	**Calibrator 1.56**	**QC POSIT.**	Sample 7	Sample 9	Sample 17	Sample 25
H	**Blank**	**QC NEGAT.**	Sample 8	Sample 10	Sample 18	Sample 26

Figure 4: Example of a work sheet.

EVALUATION OF THE INHIBITION TEST

To determine if a sample is a “true positive”, compare the measured levels of IgG anti-IgA antibodies in the two different preparations for each sample (sample + Inhibitory Control POSITIVE, sample + Inhibitory Control NEGATIVE).

If the measured levels of IgG anti-IgA in the sample are not a false positive, then the levels of these antibodies should decrease in the sample containing the Inhibitory Control POSITIVE (e.g. 50% or more; refer to Certificate of Analysis) in comparison to the level from the corresponding sample measured with the Inhibitory Control NEGATIVE. The rate of decline is due to the ratio of IgA in the Inhibitory Control POSITIVE and anti-IgA antibodies in the tested sample.

16. COMPARISON OF UNITS U/ml WITH PREVIOUSLY USED TITRE EVALUATION

Human Anti-IgA isotype IgG ELISA from BioVendor Company was compared with the other ELISA test (competitor's test), which used the titre evaluation. Comparison of the results of the two methods was evaluated by linear regression:

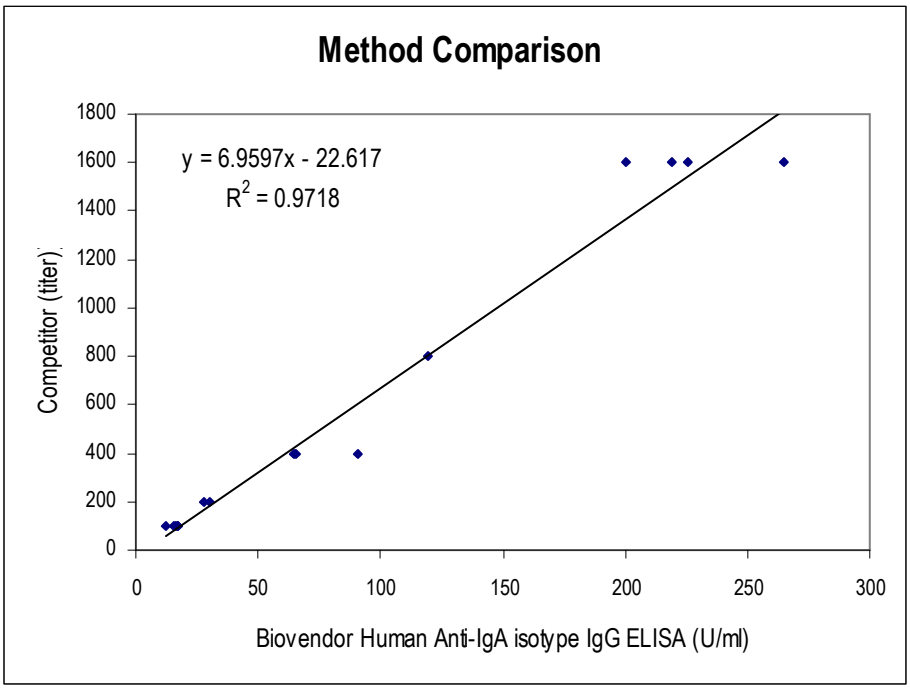


Figure 5: Method comparison.

Cut-off limit (see Chapter 12) set in our ELISA titer corresponds to **1:100**. Customer is allowed the possibility to measure in titres with a single warning that the converted final titre must be read only for samples with values over or equal to the cut-off threshold. The levels read under the cut-off limit are not clinically relevant and are considered to be negative.

17. TROUBLESHOOTING AND FAQS

›› Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

›› High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 40° C

›› High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Calibrators, Quality Controls or samples

18. REFERENCES

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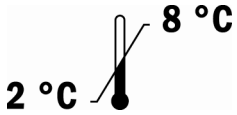
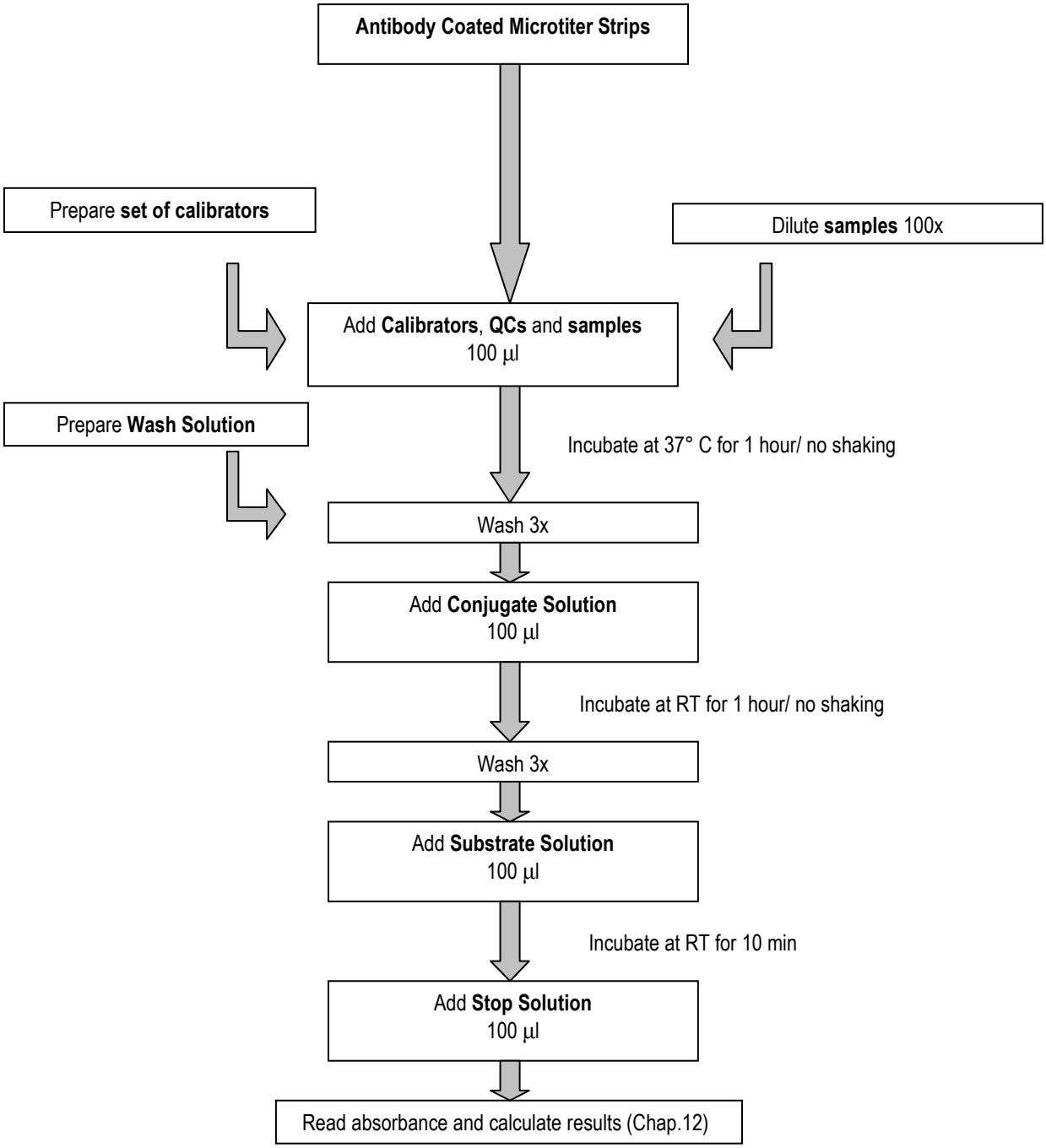
19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary



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A												
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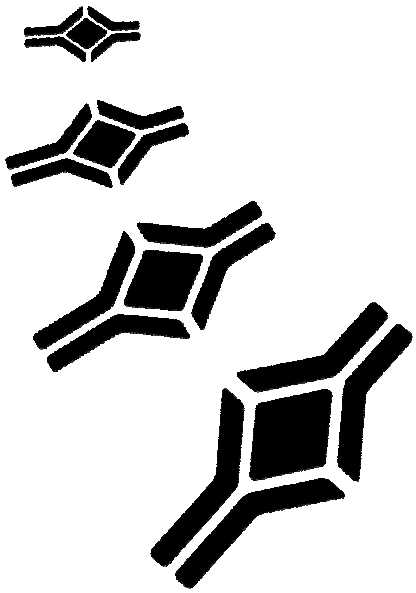

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