

Anti-t. gondii igg antibody measurement



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VIDAS Method for the Measurement Anti-T. gondii IgG antibody**Principle:**

The assay principle a two-step enzyme immune assay sandwich methods with a final fluorescent detection (ELFA). The solid phase Receptacle (SPR) has been used as the solid phase also as pipetting device for the assay. Reagent for the assay have been ready-to-used and pre-dispensed in the sealed reagent strips. The assay steps have been performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. After a sample dilution step, the sample is cycled in and out the SPR. Anti-T. gondii IgG antibodies present in the spacemen will bind to the T. gondii antigen coating the anterior of the SPR. Unbound component are eliminated during the washing steps. Mouse monoclonal anti-human IgG conjugate with alkaline phosphatase is cycled through the SPR and will attach to any human IgG bound the SPR wall. During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled and out of the SPR. The conjugate enzyme catalyse the (4- Methyl-umbellefrone) the fluorescent of which is measured at 450 nm. The intensity of the fluorescence of which is proportional to the concentration of antibodies present in the sample. At the end of the assay, result are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out[237]

Content of the kit (60 tests): 3. 4. 1. 2

Ready-to-use

STR

60 TXG

		strips
Ready-to-use.		
SPRs coated with membrane and cytoplasmic toxoplasma antigen, RH Sabin strain grown in mice (12).	SPR	60 TXG SPRs 2X30
Human serum* containing anti-Toxoplasma IgG protein stabilizer + 1 gl sodium azide.		TXG positive
Titer in IU/ml is indicated on MLE card after the following mention: “ control C1 (+) dose value range”.	C1	control 1X2 ml (liquid)
Human serum* negative for anti-Toxoplasma IgG protein stabilizer + 1 gl sodium azide.	C2	TXG negative control 1X3 ml (liquid)
Human serum* containing anti-Toxoplasma IgG and calibrated against the 2nd WHO	S1	TXG calibrato r

International standard +
protein stabilizer + 1 gI
sodium azide.

Titer in IUml : the

concentration in IUml is

indicated on the MLE card

1X1 ml

after the following mention: “

(liquid).

calibrator (S1) dose value”.

The confidence interval in “

relative fluorescence value” is

indicated on MLE card after

the following mention: ”

calibrator (S1) RFV Rang.

Specification for the factory

1 MLE

master data required two

card

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1 package insert

*this product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However , signs no existing test method can totally guarantee their absence, this product must be treated as potentially infectious, therefore, usual safety procedures should be observed when handling.

<https://assignbuster.com/anti-t-gondii-igg-antibody-measurement/>

The solid phase receptacle (SPR)

The interior of the SPR is coated during production With membrane and cytoplasmic toxoplasma antigen(RH sabin strain). Each SPR is identified by TXG code.

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, Kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of sample. The last well of each strip is acuvette in which the flourimetric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the TXG strip**Well Reagents**

1 Sampel well.

Serum diluents: TRIS buffer (50mmol/l) PH

2 7. 4+protein and chemical stabilizer+0. 9 g/l sodium azide (600µl).

Pre-wash buffer: TRIS buffer (50mmol/l)

3 PH 7. 4+protein and chemical stabilizer+0. 9 g/l sodium azide (600µl).

4-5- Wash buffer: TRIS buffer (50mmol/l) PH 7.

7-8 4+protein and chemical stabilizer+0. 9 g/l

sodium azide (600µl).

Conjugate: Alkaline phosphatase-labeled

6 monoclonal anti-human IgG Antibodies
(mouse) + 0.9g/l sodium azide (400 µl).

Serum diluents: TRIS buffer (50mmol/l) PH

9 7.4+ protein and chemical stabilizer + 0.9
g/l sodium azide (400µl).

Cuvette with substrate: 4-Methyle-

10 umbelliferyl phosphate (0.6mmol/l)
+diethanolamine(DEA) (0.62mol/l or 6.6%,
PH 9.2)+1g/l sodium azide (300µl).

Procedure

1. The required reagents were removed from the refrigerator and allowed to come to room temperature for at least 30 minutes.
2. Used one "TXG" strip and one "TXG" SPR for each sample, control or calibrator to be tested.
3. The test is identified by the "TXG" code on the instrument. The calibrator must be identified by "S1" and tested in duplicate. If the positive control is to be tested it should be identified by "C1". If the negative control needs to be tested, it should be identified by C2.
4. Mix the calibrator, control and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
5. For this test, the calibrator, control, and sample test portion is 100µl.

6. Insert “ TXG” SPR S and “ TXG” strips into the instrument. Check to make sure the color labels with the assay code and the SPR S and Reagent strips match.
7. Initiate the assay as directed in the user’s manual. All the assay steps are performed automatically by the instrument.
8. Re stopper the vials and return them to 2-8 °C after pipetting.
9. the assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPRs and strips from the instruments.
10. Dispose of the used SPRs and strips into an appropriate recipient.

Results and interpretation:

Once the assay is completed, results are analyzed automatically by the computer fluorescence is measured twice in the Reagent Strip’s reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (relative fluorescence value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

Titer (IU/ml)	Interpretati on
<4	Negative
4 ≤ Titer <8	Equivocal

≥8 Positive

The results are automatically calculated by the instrument using calibration curves which are stored by the instrument (4-parameter logistic model) and are expressed in (IU/ml) (2nd WHO international standard).

VIDAS Method for the Measurement Anti-T. gondii IgM antibody:

Principle:

The assay principle a two-step enzyme immune assay sandwich methods with a final fluorescent detection (ELFA). The solid phase Receptacle (SPR) has been used as the solid phase as well as pipetting device for the assay. Reagent for the assay have been ready-to-used and pre-dispensed in the sealed reagent strips. The assay steps have been performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. After a sample dilution step, the IgM are captured by polyclonal Ab coating the interior of the SPR. Anti-T. gondii IgM are specifically detected by inactivated toxoplasma antigen (RH sabin strain), which is itself revealed by an alkaline phosphatase-labeled murine monoclonal anti body (anti-p30). During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbellifrone) the fluorescent of which is measured at 450 nm. The intensity of the fluorescence of which is proportional to the concentration of antibodies present in the sample. At the end of the assay, result are automatically calculated by the instrument in relation to the S1 standard stored in memory, and then printed out.

Content of the kit (60 tests): 3. 4. 2. 2

Ready-to-use	STR	60 TXM strips
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Ready-to-use.		60 TXM
Interior of SPR coated with anti-human μ chain antibodies (goat).	SPR	SPRs 2X30

Human serum* containing anti-Toxoplasma IgM protein stabilizer + 1 gl sodium azide.		TXM positive
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Index : the confidence interval is indicated on MLE card after the following mention: “ control C1 (+) test value range”.	C1	control 1X2 ml (liquid)
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Human serum* negative for anti-Toxoplasma IgM protein stabilizer + 1 gl sodium azide.	C2	TXM negative control 1X2 ml (liquid)
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Human serum* containing	S1	TXM
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anti-Toxoplasma IgM	Standard
+ protein stabilizer + 1 gl	1X1 ml
sodium azide.	(liquid).

Specification for the factory	1 MLE
master data required two	card
calibrate the test : to read	(master
MLE data.	lot
	entery)

1 package insert

*this product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However , signs no existing test method can totally guarantee their absence, this product must be treated as potentially infectious, therefore, usual safety procedures should be observed when handling.

The solid phase receptacle (SPR)

The interior of the SPR is coated during production With anti-human μ chain antibodies . Each SPR is identified by TXM code.

The strip

The same as of the strip T. gondii IgG.

Description of the TXM strip

Well Reagents

<https://assignbuster.com/anti-t-gondii-igg-antibody-measurement/>

1 Sampel well.

Serum diluents: TRIS buffer (50mmol/l) PH

2 7. 4+protein and chemical stabilizer+0. 9
g/l sodium azide (300µl).

Pre-wash buffer: TRIS buffer (50mmol/l)

3 PH 7. 4+protein and chemical
stabilizer+0. 9 g/l sodium azide (600µl).

4-5- Wash buffer: TRIS buffer (50mmol/l) PH 7.
7-8 4+protein and chemical stabilizer+0. 9 g/l
sodium azide (600µl).

6 Conjugate: Alkaline phosphatase-labeled
immune complex (toxoplasma antigen RH
sabin strain grown in mice(12)-mouse
monoclonal anti-p30antibodies) + 0. 9g/l
sodium azide+0. 02%gentamicine (400
µl).

9 Empety well

10 Cuvette with substrate: 4-Methyle-
umbelliferyl phosphate (0. 6mmol/l)
+diethan

olamine(DEA) (0.62mol/l or 6.6%, PH 9.

2)+1g/l sodium azide (300µl).

Procedure

The same as procedure of T. Gondii IgG Ab.

Results and interpretation

Once the assay is completed, results are analyzed automatically by the computer fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (relative fluorescence value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet. The instrument calculate a test value for each sample (index), which is the ratio between its RFV and that the memorized standard . Interpretation of test result should be made by taking into consideration the patient's history , and the results of any other tests performed or other IgM assay methods.

Index	Interpretation
$I < 0.55$	Negative

$0.55 \leq i < 0.65$ Equivocal

$i \geq 0.65$ Positive

3. 4. 3VIDAS Method for the Measurement Anti CMV IgG antibody:

3. 4. 3. 1 principle

The same as principle Anti-toxoplasma IgG method .

3. 4. 3. 2Content of the kit (60 tests):

Ready-to-use	STR	60 CMVG strips
Ready-to-use.		60 CMVG
SPRs coated withCMV antigen (strain AD 196) .	SPR	SPRs 2X30
Human serum* containing anti-CMV IgG + 1 gl sodium azide.	C1	CMVG positive control 1X1. 5 ml
Titer in arbitrary unit /ml(aU/ml) : confidence interval is indicated on the MLE card after the following mention “ control C1 (+) dose		(liquid)

value range”.

	CMVG
Human serum not	negative
containing anti-CMV IgG C2	control
+ 1 gl sodium azide.	1X1. 5 ml
	(liquid)

Human serum*
containing anti-CMV IgG
+ 1 gl sodium azide.

Titer in arbitrary unit/ml

indicated on the MLE

card after the following

mention: “ calibrator

(S1) dose value”. The S1

confidence interval in “

Relative fluorescence

value” is indicated on

MLE card after the

following mention: ”

calibrator (S1) RFV

Rang.

CMVGcalibrat

or

1X2 ml

(liquid).

Specification for the	1 MLE
factory master data	card

required two calibrate (maste
the test : to read MLE r lot
data. entery)

1 package insert

The solid phase receptacle (SPR)

The interior of the SPR is coated during production With purified CMV antigen. Each SPR is identified by CMVG code.

The reagent strips

The same as reagent strip of the anti- toxo IgG that explained previously.

Description of the CMVG strip

Well Reagents

1 Sampel well.

sample diluents: phosphate buffer

2 (10mmol/l) PH 7. 2-Tween +protein and
chemical stabilizer+ (300µl).

Pre-wash buffer: phosphate buffer

3 (10mmol/l) PH 7. 2-Tween +protein and
chemical stabilizer+1g/l sodium azide
(600µl).

4-5- Wash solution: TRIS buffer (50mmol/l) PH

7-8 7.4 + 0.9 g/l sodium azide (600µl).

Conjugate: Alkaline phosphatase-labeled

6 monoclonal anti-human IgG antibodies
(mouse) + 1g/l sodium azide (400 µl).

9 Empty well

Cuvette with substrate: 4-Methyle-
10 umbelliferyl phosphate (0.6mmol/l)
+ diethanolamine (DEA) (0.62mol/l or 6.6%, PH 9.2) + 1g/l sodium azide (300µl).

Procedure

The same as procedure of T. Gondii IgG Ab.

Results and interpretation

The results measured as the same as method to Toxo-IgG Ab that explained previously. But if the sample with anti-CMV > 400 aU/ml should be re-assayed after dilution by 1/4 in saline solution. If the dilution factor has not been entered when the work list was created, multiply the result by dilution factor to obtain the sample concentration.

Thresholds and interpretation of results

value (IU/ml)	Interpretati on
< 4	Negative

From ≥ 4
< 6 Equivocal

≥ 6 Positive

VIDAS Method for the Measurement Anti-CMV IgM antibody:

Principle:

The assay principle a two-step enzyme immune assay sandwich methods with a final fluorescent detection (ELFA). The solid phase Receptacle (SPR) has been used as the solid phase as well as pipetting device for the assay. Reagent for the assay have been ready-to-used and pre-dispensed in the sealed reagent strips. The assay steps have been performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times. After IgG and rheumatoid factor adsorption , the sample is cycled in and out the SPR for a specified length of time. Anti-CMV IgM antibodies present in the spacemen will bind to the CMV antigen coating the anterior of the SPR. Unbound component are eliminated during the washing steps. An alkaline phosphatase-labeled mouse monoclonal anti-human IgM antibody is cycled in and out of SPR. A final wash step removes unbound component. During the final detection step, the substrate (4- Methyl-umbelliferyl phosphate) is cycled and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4- Methyl-umbellefrone) the fluorescent of which is measured at 450 nm. The intensity of the fluorescence of which is proportional to the concentration of

antibodies present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out [238, 239 and 240].

Content of the kit (30 tests): 3. 4. 4. 2

Ready-to-use.	STR	30 CMVM strips
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Ready-to-use.

Interior of SPRs coated with CMV antigen (cell culture of the virus of the AD 196 strain) .	SPR	30 CMVM SPRs
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Ready-to-use.

Human serum* containing anti-CMV IgM + 1 gl sodium azide. Index:		CMVM positive
The confidence interval is indicated on the MLE card after the following mention : “ control C1 (+) dose value range”.	C1	control 1X0. 5 ml (liquid)

Ready-to-use.	C2	CMVM negative
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Human serum not	control
containing anti-CMV IgM +	1X1 ml
1 gl sodium azide.	(liquid)
Ready-to-use.	CMVM
Human serum* containing	calibrator
anti-CMV IgM + 1 gl	1X1ml
sodium azide.	(liquid).

Specification for the	1 MLE
factory master data	card
required two calibrate the	(master
test : to read MLE data.	lot
	entery)

1 package insert

The solid phase receptacle (SPR)

The same as SPR of CMV IgG that explained previously.

The reagent strip

The same as reagent strip of CMV IgG that explained previously.

Description of the CMVM strip

Well Reagents

- 1 Sampel well.
- 2 IgG and rheumatoid factor adsorbant(anti-

human IgG goat serum) +1g/l sodium azide (300µl).

IgG and rheumatoid factor adsorbant(anti-

3 human IgG goat serum) +1g/l sodium azide (600µl).

Pre-wash solution : phosphate (10mmol/l)

4 PH 7. 2+Tween +protein and chemical stabilizer+1 g/l sodium azide (600µl).

5-7- Wash solution: TRIS buffer (50mmol/l) PH

8-9 7. 4 +0. 9 g/l sodium azide (600µl).

Conjugate: Alkaline phosphatase-labeled

6 monoclonal anti-human IgM antibodies (mouse) + 1g/l sodium azide (400 µl).

Reading cuvette with substrate: 4-

Methyle-umbelliferyl phosphate (0.

10 6mmol/l)+diethanolamine(DEA) (0.

62mol/l or 6. 6%, PH 9. 2)+1g/l sodium azide (300µl).

Procedure

The same as procedure of CMV IgG but this assay will be completed within approximately 60 minute.

Results and interpretation

The results measured as the same as method to CMV-IgG Ab that explained previously.

Thresholds and interpretation of results

Index	Interpretation
$I < 0.07$	Negative
$0.07 \geq i < 0.09$	Equivocal
$I \geq 0.09$	Positive

The data was put on computer file for storage and analysis. Descriptive statistics included the use of frequencies, relative frequencies, means, standard deviations and ranges. The Chi-Square statistical test was used to test for associations between variables with the results being considered as statistically significant when the P value was ≤ 0.05 . SPSS statistical package version 20 was used for data description and analysis. Fisher exact test or Yates correction formula was applied whenever applicable.

Mean	Range	Standard Error of Mean	Standard Deviation	Minimum	Maximum
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	n					
AGE	26.	27.	0.	6.55	16.00	43.00
	21	00	42			