



PICTOR LIMITED

ACCESSIBLE DIAGNOSTIC TECHNOLOGY
FOR A HEALTHY WORLD

PICTARRAY™

TORCH

Instructions

PictArray™ ToRCH ELISA Kit

Instructions for testing human serum samples on PictArray™ ToRCH Panel consisting of *Toxoplasma gondii*, Rubella, Cytomegalovirus (CMV), Herpes Simplex Virus (HSV) 1 and 2

PLEASE READ COMPLETE INSTRUCTIONS BEFORE USING THIS KIT

1 >>> SIMPLE



2 >>> AFFORDABLE



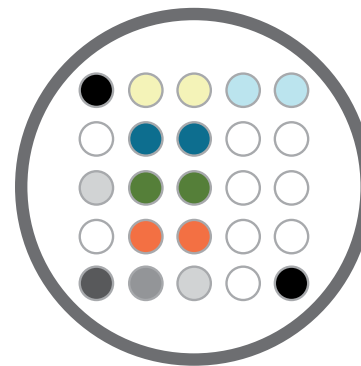
3 >>> ACCURATE








1. Intended Use

PictArray ToRCH Panel is a miniaturized and multiplexed immunoassay for detection of IgG and IgM antibodies in human sera to *Toxoplasma gondii*, Rubella, CMV, HSV1 and HSV2. This *in vitro* test is intended as an aid in detecting exposure and infection with any of the five ToRCH pathogens. PictArray results can be reported either for individual tests or for the complete panel.

Layout for PictArray ToRCH Panel



Spot	Antigen
	<i>T. gondii</i>
	Rubella
	CMV
	HSV 1
	HSV 2

2. Summary and explanation of the test

Infection with one of the ToRCH pathogens contracted during pregnancy may be passed through the placenta to the fetus, affecting the fetus and newborn potentially causing serious birth defects. Asymptomatic infants may develop abnormalities later in life. ToRCH screening is now widely requested by clinicians investigating infants and pregnant women for congenital, perinatal and neonatal infections.

PictArrays provide an efficient method to screen patients for infection with ToRCH pathogens by simultaneously testing reactivity to any one or all five pathogenic antigens. The analysis of test data by computer-generated algorithms reduces subjectivity in interpretation of results. Internal controls



are included in every test to monitor performance reducing the chances of human and mechanical errors as compared to a conventional ELISA assay done using one well-one test microtiter plates.

3. Test principle

The detection of antibodies to pathogenic antigens is based on the ELISA principle.

- a. Diluted serum samples are incubated in wells containing duplicate spots of five different pathogenic antigens and nine test controls.
- b. After washing non-bound serum components, binding of IgG or IgM is detected by sequential incubations either with biotin-labelled anti-human IgG or anti-human IgM, followed by a Streptavidin-peroxidase conjugate.
- c. The free conjugates are washed off and enzymatic activity bound to the antigen spots is measured by incubation with substrate.
- d. The reaction is stopped by washing off excess substrate and the slide is imaged. The color intensity of each spot is directly proportional to the amount of antibody bound to the antigen.

4. Kit components

Component	Tube Label	Description		
PictArray slides		Test slide with 8 two-well modules containing 300 μ spots of test reagents	1 slide	3 slides
Wash Buffer 10X	WASH	Buffer containing phosphate buffered saline with Tween 20	4mL	12mL
Diluent 10X	DIL	Buffer containing blocking agent and phosphate buffered saline with Tween 20	1.1mL	7mL
IgM Assay Diluent 4X	DIL M	Buffer containing reagents to reduce interference from serum components	1.1mL	4ml
Conjugate G 20X	CONJ G	Anti-human IgG antibody conjugated to biotin	0.15mL	0.3mL
Conjugate M 20X	CONJ M	Anti-human IgM antibody conjugated to biotin	0.15mL	0.3mL
Detection reagent 20X	DET	Streptavidin conjugated to horseradish peroxidase	0.15mL	0.3mL
Substrate A 20X	SUBS A	Solution containing diaminobenzidine tetrahydrochloride	0.15mL	0.3mL
Substrate B 1X	SUBS B	Buffer containing Hydrogen peroxide	2mL	6mL
Pack insert		Protocol for using the PictArray ToRCH ELISA test kit	1	1

5. Storage conditions

Store the unopened kit immediately upon arrival at 4 °C. DO NOT FREEZE.



6. Materials required but not provided

6.1 For laboratories manually processing moderate numbers of samples

- Deionized or distilled water
- Precision pipettes to deliver from 5 µl to 200 µl volumes.
- Adjustable 1 ml pipettes for reagent and sample preparation.
- Incubator set at 37 °C.
- PictImager for reading test results
- Computer installed with Pictorial Image Analysis software.

6.2 For laboratories with automated sample processing flow

NOTE:

A specially-designed frame to hold up to four 16-well slides is available from Pictor.

This reusable frame fits into most commercially available ELISA plate processors

- Deionized or distilled water
- Automated ELISA plate washers or analyzers programmed to process PictArray slides.
- PictImager for reading test results
- Computer installed with Pictorial Image Analysis software.

7. Precautions

7.1 Safety precautions

- All reagents in this kit are for *in vitro* diagnostic use only.
- Handle all human material as if potentially infectious. Users should wear gloves and protective clothing when handling any patient sera or serum-based products.
- Avoid any contact of the reagents in the kit with the skin and mucosa (toxicity, irritation and burn hazard). Material safety data sheets for kit components are available upon request.

- Handle and dispose samples and reagents in accordance with local legislation and established laboratory protocols.

7.2 Technical precautions

- Follow complete instructions for proper performance of the kit and to obtain reliable results.
- Ensure that the slide and/or the two-well modules are correctly aligned with the sample layout before adding samples.
- Only freshly drawn and properly refrigerated sera should be used in this assay. Sera may be stored between 2 °C and 8 °C for no longer than 48 hours. If delay in testing is anticipated, the sera should be stored at -20 °C.
- Do not allow the wells in the slide to dry between any of the steps during sample processing until the final wash step is performed.
- It is important to add samples and reagents into wells without any delay. Please ensure that all the samples and reagents are ready to dispense.
- Use a different pipette tip for each sample and change tips for addition of different reagents. Cross contamination of reagents and/ or samples can lead to incorrect results.

8. Preparation of Reagents

NOTE:

Volumes below are sufficient to process one slide. Volumes should be increased proportionally to test more slides.

Keep undiluted stock reagents at 4°C immediately after use.

Diluted 1X Wash Buffer, 1X Diluent and 1X IgM Assay Diluent are stable for 3 months when stored at 4°C.

All other diluted 1X reagents have to be used within 2 hours.



1. **Spin all tubes in the kit briefly before use.**

2. **1X Wash Buffer (WASH)**

NOTE:

If crystals are visible, warm the tube at 37 °C for 10 minutes.

Add 4ml of 10X Wash Buffer into a 100ml cylinder.

Add distilled water to make volume to 40ml.

Mix well and transfer into a bottle labelled “1x Wash Buffer”.

Store at 4 °C.

3. **1X Diluent (DIL)**

Add 1ml of 10X Diluent into a 10ml cylinder.

Add 9ml of 1X Wash Buffer to make volume to 10ml.

Mix well and transfer into a labelled “1X Diluent” 10mL tube.

Store at 4 °C.

4. **1X IgM Assay Diluent (DIL M)**

Add 1.0ml of 4X IgM Assay Diluent (DIL M) into a 10ml screw cap tube labelled “1X Diluent”.

Add 3.0ml of 1X Diluent to make volume to 4.0ml.

Mix well and store at 4 °C.

9. **Test Protocol**

NOTES:

1. **2-well modules may be processed individually. Unused modules should be kept refrigerated.**
2. **Please refer to “Custom Sample Layout” section in the Pictorial Image Analysis Manual**

3. **The top of the slide has a notch on the upper left corner which is used as an alignment reference.**

1. **Sample Layout**

Please follow instructions in the Pictorial© Image Analysis Instruction Manual to generate sample layout.

2. **Preparation of human serum samples**

(a) For IgG detection

Label one set of tubes depending on the number of samples to be tested.

Add 45µl of Diluent to each tube.

Add 5µl of serum sample to each tube starting from Tube 1.

Vortex gently and spin briefly.

This gives a 10-fold dilution of each sample.

Label a second set of tubes.

Add 95µl of Diluent to each tube.

Add 5µl of 10-fold diluted serum sample to each tube starting from Tube 1.

Vortex gently and spin briefly.

This gives a 200-fold dilution of each sample.

(b) For IgM detection

Label one set of tubes depending on the number of samples to be tested.

Add 45µl of IgM Assay Diluent (DIL M) to each tube.

Add 5µl of serum sample to each tube starting from Tube 1.

Vortex gently and spin briefly.

This gives a 10-fold dilution of each sample.

Label a second set of tubes.

Add 90µl of IgM Assay Diluent (DIL M) to each tube.

Add 10µl of 10-fold diluted serum sample to each tube starting from Tube 1.

Vortex gently and spin briefly.

This gives a 100-fold dilution of each sample.

3. Addition of human serum samples to PictArrays

Remove the required number of modules and attach to the slide backing.

Touch only the corner of the wells with the pipette tips to avoid damaging the well surface.

Ensure that the notch on the upper left side of the slide aligns with the layout.

Add 50µl of the appropriately diluted sample to array wells according to the printed layout.

NOTE:

Add Diluent to the well not containing sample, if single sample is to be tested.

Place the slide in the incubation chamber during incubation to prevent drying of liquid.

Incubate the slide at 37 °C for 30min.

Aspirate the samples.

Wash each well with 50µl of Wash Buffer.

Repeat the washes twice more.

4. Addition of Conjugate (CONJ G or CONJ M)

Prepare the appropriate volume of 1X CONJ G and/or 1X CONJ M to measure IgG or IgM antibodies as shown in the table below:

Number of wells	1X Diluent(µl)	20X Conjugate (µl) (CONJ G or CONJ M)
2	200	10
4	300	15
6	400	20
8	500	25
10	600	30
12	700	35
14	800	40
16	900	45

Add 50µl of either Conjugate G or Conjugate M for detection of ToRCH IgG or IgM, respectively to the wells as indicated in the layout.

Place the slide in the incubation chamber.

Incubate the slide at 37 °C for 30min.

Aspirate the conjugate.

Wash each well with 50µl of Wash Buffer.

Repeat the washes twice more.

5. Addition of Detection Reagent (DET)

Prepare the detection reagent prior to use as shown in the table below:

1X DET has to be used within 60 mins of preparation.

Number of wells	1X Diluent (μl)	20X Detection Reagent (μl) (Streptavidin-horseradish peroxidase)
2	200	10
4	300	15
6	400	20
8	500	25
10	600	30
12	700	35
14	800	40
16	900	45

Add 50 μl of the prepared Detection Reagent to each well.

Place the slide in the incubation chamber.

Incubate the slide at 37 °C for 30min.

Aspirate the Detection Reagent.

Wash each well with 50 μl of Wash Buffer.

Repeat the washes twice more.

6. Addition of Substrate Solution (SUBS)

Prepare the substrate no more than 30 mins prior to use as shown in the table below:

Number of wells	1X Substrate B (μl)	20X Substrate A (μl)
2	200	10
4	300	15
6	400	20
8	500	25
10	600	30
12	700	35
14	800	40
16	900	45

Add 50 μl of the diluted Substrate Solution to each array well.

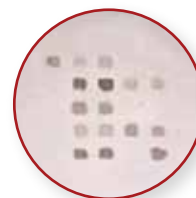
Incubate the slide at room temperature for 5min.

NOTE:

NOTE: Please monitor color development closely. Brown spots begin to appear in the arrays and the background begins to turn orange.

Allow the reaction to continue longer than 5min based on the signal to noise ratio of spot intensity to background. Incubate the slide for an additional 2 to 3 minutes, if necessary.

An example of a typical array is shown alongside



7. Stopping the Reaction

Aspirate off the Substrate Solution.

Wash each well with 50µl of Wash Buffer to stop the reaction.

Aspirate off the Wash Buffer.

8. Scanning and Analysis

Dry the slide at 37 °C for 30min.

Read the slide following instructions in the “Pictorial© Image Analysis Manual”.

10. Results

Pictorial Image Analysis software measures the color intensity values for each of the array spots.

The intensity values of test spots are corrected against the intensity values from background and control spots using proprietary algorithms.

1. Quality Control

- Control spots in each well are intended to monitor for substantial reagent failure.
- Additional controls may be tested according to guidelines or requirements of local regulatory agencies.
- Measurement of intensity values of Control spots embedded in each PictArray well ensures that the test has been performed correctly.

The test is valid if the performance control and sample control show acceptable intensity values. If these criteria are not met, the test results are reported as an “Error” by Pictorial software.

2. Units

The test results for patient samples are reported as Internal Unit/ ml defined as the increase in IgG and IgM antibody binding to the ToRCH antigens compared to healthy subjects.

For example, a value of 10IU/ ml is a 10-fold increase in antibody levels compared to non-diseased samples.

The cut-off value of 0.8 IU/ ml was obtained for each pathogen by testing serum samples obtained from healthy donors with no detectable antibody binding to ToRCH antigens on the array.

3. Measuring range

PictArrays generate a quantitative result by measuring the increase in intensity values obtained upon binding of IgG and IgM antibodies from patient serum to the ToRCH antigens.

Negative	less than 0.8 IU/ ml
Borderline	0.81 to 1.3 IU/ ml
Weak Positive	1.31 to 2.5 IU/ ml
Positive	greater than 2.51 IU/ ml

NOTE:

Results reported as borderline (+/-) should be interpreted with caution. These results occur when the IU/ ml values are in the grey area which, if the test is repeated, could result in either a positive or a negative outcome.

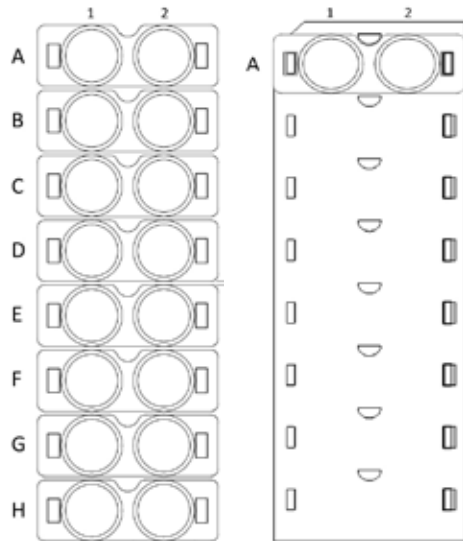


11. Limitations

- This is a semi-quantitative test and the units when given as IU/ ml are provided as a guide to relative increase in levels compared to a healthy population.
- Repeated freeze-thaw of the sample should be avoided.
- The diagnosis should not be made solely on the basis of PictArray ToRCH panel test results.
- Test results should be interpreted in conjunction with clinical evaluation and the results of other diagnostic procedures.
- PictArray ToRCH panel performance has not been tested against lipemic and hemolyzed samples, and those containing high levels of bilirubin.

12. PictArray format

2-well unit layout





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