ROSS RIVER VIRUS ELISA IgG

For *in vitro* diagnostic use

G1011: Indirect immunoenzyme assay to test IgG antibodies against Ross River Virus in human serum/plasma. 96 tests.

INTRODUCTION:

Ross River Virus (RRV) is an Alphavirus (Togaviridae family) endemic to Australia, Papua New Guinea and other islands on the Pacific. RRV is transmitted by mosquitoes including several species in the Aedes and Culex genera. Kangaroos and wallabies are thought to be the main animal reservoirs, although domestic and peridomestic animals can be involved in transmission in urban areas. Most common symptoms of RRV disease include fever, polyarthralgia and rash; yet, more than half of the infections are subclinical. Most patients recover within a few weeks, but some develop chronic disease that may last for months to years. The average incubation period is 7-9 days. Diagnosis is usually made via serology since viremia is rarely present at the onset of symptoms. Antibodies can be detected as early as 2-3 days after the onset of symptoms. Hemagglutination inhibition and neutralization methods can be used for serological diagnosis, but other methods that are capable of specifically detecting IgG or IgM responses, such as enzyme immunoassays, are preferred. Persistent IgM antibodies to RRV have been detected up to 2 years after the acute infection.

PRINCIPLE OF THE TEST:

The ELISA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution.

KIT FEATURES:

All reagents, except for the washing solution, are supplied ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Sample predilution is not necessary.
Break-apart individual wells are supplied, so that the same number of wells is consumed than the number of tests performed.

KIT CONTENTS:

1 VIRCELL ROSS RIVER VIRUS IgG PLATE: 1 96-wells plate coated with RRV antigen (strain NCPV0005281v)).
2 VIRCELL SERUM DILUENT: 25 ml of serum dilution solution: a blue coloured phosphate buffer containing protein stabilizers and Neolone and Bronidox. Ready to use.
3 VIRCELL IgG POSITIVE CONTROL: 500 µl of positive control serum containing Neolone and Bronidox.
4 VIRCELL IgG CUT OFF CONTROL: 500 µl of cut off control serum containing Neolone and Bronidox.
5 VIRCELL IgG NEGATIVE CONTROL: 500 µl of negative control serum containing Neolone and Bronidox.
6 VIRCELL IgG CONJUGATE: 15 ml of anti-human IgG peroxidase conjugate dilution in an orange-coloured Neolone and Bronidox-containing buffer. Ready to use.
7 VIRCELL TMB SUBSTRATE SOLUTION: 15 ml of substrate solution containing tetramethylbenzidine (TMB). Ready to use.
8 VIRCELL STOP REAGENT: 15 ml of stopping solution: 0.5 M sulfuric acid.
9 VIRCELL WASH BUFFER: 50 ml of 20x washing solution: a phosphate buffer containing Tween^{R}-20 and Proclin 300.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:
-Precision micropipettes 5 and 100 µl.
-Eight channel micropipette 100 µl.
-ELISA plate washer.
-Thermostatized incubator/water bath.
-ELISA plate spectrophotometer with a 450 nm measuring filter and a 620 nm reference filter.
-Alternatively, an ELISA automated processor.
-Distilled water.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
1x washing solution	4 months at 2-8ºC
Rest of reagents	Refer to package label for expiration date (at 2-8ºC)

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.
Do not let the plate dry between washing and reagent addition.
Substrate solution is light sensitive. Avoid light exposure and discard if blue colour develops during storage. Substrate solution should not get in contact with oxidizers such as bleach solutions or metals. Make sure that no metal components come in contact with the substrate.
Use only the amount of washing, serum dilution, conjugate and TMB solutions required for the test. Do not return the excess solution into the bottles.

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only the serum dilution, washing, stopping and substrate solutions are compatible with the equivalents in other VIRCELL ELISA references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Do not use in the event of damage to the package.
5. Never pipette by mouth.
6. Serum dilution solution, plate, conjugates and controls in this kit include substances of animal origin. Controls include as

well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. The wells are coated with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that these or other infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.

7. Before incorporating this product onto an automatic processing system, we strongly recommend the performance of a pre-evaluation assay. To this purpose, VIRCELL counts with sets of samples reserved for evaluation in parallel with the manual technique. These sets of samples are available on request, as well as a list of commercial systems which have already been validated for use with the VIRCELL ELISA range.

8. During incubation times, an adequate sealing of the plates with the adhesive film included in the kit avoids the desiccation of the samples, and guarantees the repeatability of the results.

9. Avoid contact of Stop Solution (0.5 M sulfuric acid) with skin or eyes. If contact occurs, immediately flush the area with water.

10. Proclin 300 is included as preservative in the washing solution. It may cause an allergic skin reaction. If on skin, wash with plenty of soap and water. For further information a Material Safety Data Sheet is available.

SPECIMEN COLLECTION AND HANDLING:

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sample. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:

Only the washing solution must be prepared in advance. Fill 50 ml of 20x washing solution up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:

1. Set incubator/water bath to 37±1ºC.
2. Bring all reagents to room temperature before use (approximately 1 hour), without removing the plate from the bag.
3. Shake all components.
4. Remove the plate 1 from the package. Determine the numbers of wells to be employed counting in four wells for the controls: two for the cut off serum and one each for the negative and positive sera. Wells not required for the test should be returned to the pouch, which should then be sealed.
5. Add 100 µl of serum diluent 2 to all wells. Add 5 µl of each sample, 5 µl of positive control 3, 5 µl of cut off control 4 (in duplicate) and 5 µl of negative control 5 into the corresponding wells. If the assay is performed manually, shake the plate in a plate shaker (2 min) in order to achieve a homogenous mixture of the reagents. If for some reason correct shaking cannot be guaranteed, a pre-dilution of the sample in a separate tube or plate should be made, using double volume of serum diluent 2 and sample. Mix homogenously with the pipette and dispense 105 µl of each diluted sample to the wells 1.
6. Cover with a sealing sheet and incubate at 37±1ºC for 45 min.
7. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution 9 per well. Drain off any remaining liquid.
8. Immediately add 100 µl of IgG conjugate solution 6 into each well.
9. Cover with a sealing sheet and incubate in incubator/water bath at 37±1ºC for 30 min.
10. Remove the seal, aspirate liquid from all wells and wash five times with 0.3 ml of washing solution 9 per well. Drain off any remaining liquid.
11. Immediately add 100 µl of substrate solution 7 into each well.
12. Incubate at room temperature for 20 minutes protected from light.
13. Add immediately 50 µl of stopping solution 8 into all wells.
14. Read with a spectrophotometer at 450/620 nm within 1 hour of stopping.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.

The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:

Positive, negative and cut off controls must be run with each test run. It allows the validation of the assay and kit.

Optical densities (O.D.) must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	O.D.
Positive control	>0.9
Negative control	<0.5
Cut off control	>0.55
	<1.5

INTERPRETATION OF RESULTS:

Calculate the mean O.D. for cut off serum.

Antibody index=(sample O.D./ cut off serum mean O.D.) x 10

Index	Interpretation
< 9	Negative
9-11	Equivocal
>11	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.

Samples with indexes below 9 are considered as not having IgG specific antibodies against RRV.

Samples with indexes above 11 are considered as having IgG specific antibodies against RRV.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

• SENSITIVITY AND SPECIFICITY:

460 serum/plasma samples were assayed with ROSS RIVER VIRUS ELISA IgG against another commercial available ELISA kit.

The results were as follows:

	Samples No.	Sensitivity (%)	Specificity (%)
IgG	460	95	98
95% C.I.		89-98	95-99

C.I. Confidence intervals

Indeterminate values were omitted from the final calculations

• INTRA-ASSAY PRECISION:

3 sera were individually pipetted 10 times each serum in a single assay performed by the same operator in essentially unchanged conditions.

The results were as follows:

Serum	N	%C.V.
PC	10	2.96
NC	10	4.40
CO	10	26.82

C.V. Coefficient of variation

• INTER-ASSAY PRECISION:

3 sera were individually pipetted on 5 consecutive days by 2 different operators. The results were as follows:

Serum	N	% C.V.
PC	10	8.91
NC	10	7.13
CO	10	18.64

C.V. Coefficient of variation

• CROSS REACTIVITY AND INTERFERENCES:

58 samples known to be positive for other microrganisms (Dengue, Chikungunya, Zika, Rubella, *Coxiella burnetii*, *Toxoplasma gondii* and *Borrelia burgdorferi*) were assayed. 20 samples known to be positive for rheumatoid factor and antinuclear antibodies were assayed.

No cross-reactivity was found with *Toxoplasma gondii* (10 samples tested), Rubella (6 samples tested), *Borrelia burgdorferi* (6 samples tested) and *Coxiella burnetii* (10 samples tested). Cross-reactivity was found with Dengue (4 from 9 samples tested), Chikungunya (4 from 7 samples tested) and Zika (6 from 10 samples tested). No interferences were found with antinuclear antibodies (10 samples tested). Interferences were found with rheumatoid factor (1 from 10 samples tested).

No interferences were found with haemolytic (8.5 g/ L Hemoglobin), lipemic (2 g / L Triglycerides), hypercholesterolemic (4 g / L Cholesterol) or icteric (6 g / L Bilirubin) samples.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

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