Precision:
The precision of the assay was evaluated by testing three different sera of eight replicates over 3 days. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	10.7%	9.4%	8.6%
Inter-assay	12.8%	10.4%	8.9%

Important Note:
1. This assay is a temperature sensitive assay. The best temperature condition for this assay is from 37°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated RLU readings.
3. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication NC, PC & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY
1. To prevent false negative and false positive IgM test results, caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit have been formulated to resolve these interferences. However, in specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.
2. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

BIBLIOGRAPHY

1. Gravell, M., P.H. Dorcett, O. Gutenson, and A.C. Ley. Detection of antibody to rubella virus by enzyme-linked immunosorbent assay. J. Infect. Dis. 136:S300, 1977.
2. Hermann, K.L. Rubella virus. Manual of Clinical Microbiology, 3rd Edition. Lennette, Balows, Hausler, Truant (ed). Chapt. 86:862, 1980.
3. Katz, S.L. Rubella (German measles). Zinssmer Microbiology, 18th Edition. Jolik, Willett, Amos (ed). Chapt. 75:1067, 1985.

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

Manufactured by:
Zephyr Biomedicals
A Division of Tulip Diagnostics (P) Ltd.
M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa- 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

An ISO 13485 Certified Company

0821/VER-01

electra™
Rubella IgM

Chemiluminescence Assay for Qualitative Detection of Rubella IgM antibody in Human Serum
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE
ELECTRA™ Rubella IgM is intended for the Qualitative detection of IgM antibodies to Rubella virus infection in human serum based on capture principle. For in Vitro Diagnostic Use only.

INTRODUCTION
Rubella is a herpes virus. Generally, rubella is considered a mild adolescence disease; however a maternal infection could be transmitted through the placenta to the fetus, causing congenital rubella. Congenital rubella may result in chronic cardiac disease, growth retardation, hepatosplenomegaly, malformations and other severe abnormalities. Children born asymptomatic may develop these abnormalities later in life. To reduce risk of such severe complications, accurate serological methods must be performed to determine the serologic status of childbearing aged women.

PRINCIPLE
ELECTRA™ Rubella IgM CLIA is for use on **ELECTRA™** analyzers. **ELECTRA™ Rubella IgM** CLIA works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.
The **ELECTRA™ Rubella IgM** CLIA assay is based on the principle of capture of these immunoglobulins by anti-human IgM monoclonal agglutinating sera coated on the solid phase. A subsequent incubation with Rubella antigen conjugated to horseradish peroxidase binds the IgM antibodies specific for the antigen and is revealed by the addition of chemiluminescence substrate. The light generated is measured in relative light units (RLU) and is proportional to the amount of IgM specific antibody in the sample. The results are read by a microwell luminometer compared in a parallel manner with calibrator and controls.

MATERIALS & COMPONENTS
Materials provided with the test kits:
1. Coated Microwells: Purified anti-human IgM agglutinating sera coated wells.
2. Sample Diluent: Ready to use.
3. Negative Control: Ready to use.
4. Positive Control: Ready to use.
5. Wash Buffer Concentrate (20X).
6. Enzyme Conjugate: Ready to use.
7. Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
8. Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.

Materials required but not provided:
1. Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
2. Disposable pipette tips
3. Distilled water
4. Disposable Gloves
5. **ELECTRA™ Analyzer**

STORAGE AND STABILITY
1. **ELECTRA™ Rubella IgM** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the color of the desiccant has changed from blue to white at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable up to one week when stored at 2-8°C.
4. Working Substrate (A+B) must be used immediately.

SPECIMEN COLLECTION
1. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.

6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

- All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
- Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate- A µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate- B µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5µl of the test samples to 200 µl of sample diluent. **(Please do not dilute Positive Control and Negative Control, they are ready for use)**. Mix well.
3. For the reagent blank, dispense 100µl of sample diluent in A1 well position, followed by negative control and positive control. (recommended in duplicates). Dispense 100µl of diluted sera into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 45 minutes at 37°C.
4. Wash each well three times by filling approximately 350µl diluted wash buffer & blot dry.
5. Dispense 100µl of enzyme conjugate to each well and incubate for 45 minutes 37°C.
6. Wash each well three times by filling approximately 350µl diluted wash buffer & blot dry.
7. Add **50** µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
8. Cover the **ELECTRA™** microplate and incubate for **10** minutes at room temperature (18-25°C) in dark.
9. Read the **ELECTRA™** micro-plate exactly at **10** minutes in **ELECTRA™ Analyzer**. If **ELECTRA™** micro-plate is not read between 10-15 minutes the test results should be considered as invalid.

Add **100**µl of sample diluent in A1 Microwell as blank

⬇

Add **100** µl **NC, PC** and **diluted samples** into the respective Microwells

⬇

Apply plate sealer and incubate for **45** minutes at **37°C**

⬇

Wash Microwells **3 times** with diluted wash buffer

⬇

Add **100** µl Enzyme conjugate in each well

⬇

Apply plate sealer and incubate for **45** minutes at **37°C**

⬇

Wash Microwells **3 times** with diluted wash buffer

⬇

Add **50** µl of working substrate in each well

⬇

Incubate for **10** Minutes at **18-25°C in dark**

⬇

Read results on **ELECTRA™** Analyzer exactly at the end of **10** minutes

RUN CRITERIA

The test run may be considered valid provided the following criteria are met:

1. The Rubella IgM Index for Negative and Positive Control should be in the range stated on the labels.

CALCULATION OF RESULTS

1. Calculate the average value of the RLU of the negative control.

2. Calculate the cutoff value using the following formula:
   Cut-Off (RLU) = 10 x Mean RLU of Negative Control.

3. Calculate the Rubella IgM Index using the following formula:
   Rubella IgM Index= Sample RLU/ Cut-Off (RLU).

INTERPRETATION OF THE RESULT

IgM Index Value	Result
IgM Index value <0.90	Negative
IgM Index value 0.91-1.1	Grey zone
IgM Index value >1.1	Positive

PERFORMANCE CHARACTERISTICS

Specificity and Sensitivity:

A total of 42 patient samples were used to evaluate specificity and sensitivity of the test. Rubella IgM test results were compared to a commercial kit results:

		Reference CLIA			
		N	E	P	Total
ELECTRA™ Rubella IgM CLIA	N	26 (D)	0	0 (B)	26
	E	0	0	0	0
	P	0 (C)	0	16 (A)	16
	Total	26	0	16	42

Sensitivity = 100%
Specificity = 100%

electra™ Chemiluminescence assay
electra™ Chemiluminescence assay
electra™ Chemiluminescence assay