ELISA kits available from ADI (see details at the web site)

Measles

Catalog#	Product Description
530-100-HMG	Human Anti-Measles IgG ELISA kit, 96 tests, Quantitative
530-110-HMM	Human Anti-Measles IgM ELISA kit, 96 tests, Quantitative
530-120-HMA	Human Anti-Measles IgA ELISA kit, 96 tests, Quantitative
530-130-MMG	Mouse Anti-Measles IgG ELISA kit, 96 tests, Quantitative
530-140-MMM	Mouse Anti-Measles IgM ELISA kit, 96 tests, Quantitative
530-150-MMA	Mouse Anti-Measles IgA ELISA kit, 96 tests, Quantitative
MESL11-A	Anti-Measles (Rubeola/Edmonston strain) Virus IgG
MESL12-M	Monoclonal Anti-Measles (Rubeola/Edmonston strain) Virus IgG
MESL15-N-500	Measles (Rubeola) Virus (Edmonston) proteins/antigen extract
RP-1612	Recombinant (E.Coli) purified Measles virus Large Polymerase (2059-2183)
RP-1613	Recombinant (E.Coli) purified Measles virus Large Polymerase (58-149)
RP-651	Recombinant (E.Coli) Measles Virus Large Polymerase (58-149)
RP-653	Recombinant (E.Coli) Measles Virus Large Polymerase (2059-2183)
RP-654	duplicate entry same as #RP-1611; Recombinant Measles Virus Nucleocapsid
RP-655	Recombinant (E.Coli) Measles Virus Hemagglutinin Mosaic (1-30,115-150,379-410)

Mumps

520-100-HMG	Human Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
520-110-HMM	Human Anti-Mumps Virus (parotitis) IgM ELISA, 96 tests, Quantitative
520-120-HMA	Human Anti-Mumps Virus (parotitis) IgA ELISA, 96 tests, Quantitative
520-130-MMG	Mouse Anti-Mumps Virus (parotitis) IgG ELISA, 96 tests, Quantitative
MUMS11-S	Anti-Mumps virus (Enders) Virus antiserum
MUMS11-SB	Anti-Mumps virus (Enders) Virus antiserum
MUMS12-M	Monoclonal Anti-Mumps virus (Enders) Virus IgG
MUMS15-N-500	Mumps virus (Enders) proteins/antigens extract
510-100-HRG	Human Anti-Rubella Virus IgG ELISA kit, 96 tests, Quantitative
510-110-HRM	Human Anti-Rubella Virus IgM ELISA kit, 96 tests, Quantitative
510-120-MRG	Mouse Anti-Rubella Virus IgG ELISA kit, 96 tests, Quantitative
510-130-MRM	Mouse Anti-Rubella Virus IgM ELISA kit, 96 tests, Quantitative

Rubella

RP-1413	Recombinant (E.Coli) Rubella Virus E1 Mosaic protein
RP-1414	Recombinant (E.Coli) Rubella Virus E2 protein
RP-1415	Recombinant (E.Coli) Rubella Virus Capsid C protein
RUBL11-A	Anti-Rubella virus (HPV77 strain) IgG, unlabeled
RUBL11-BTN	Anti-Rubella virus (HPV77 strain) IgG-Biotin conjugate
RUBL11-FITC	Anti-Rubella virus (HPV77 strain) IgG-FITC conjugate
RUBL11-HRP	Anti-Rubella virus (HPV77 strain) IgG-HRP conjugate
RUBL12-M	Monoclonal Anti-Rubella virus (HPV72) E2 IgG, aff pure
RUBL13-M	Monoclonal Anti-Rubella virus envelop protein E1 IgG, aff pure
RUBL14-M	Monoclonal Anti-Rubella virus envelop protein E2 IgG, aff pure
RUBL15-M	Monoclonal Anti-Rubella virus capsid protein IgG, aff pure
RUBL15-N-500	Rubella virus (HPV77 strain) proteins/antigens extract
RUBL16-M	Monoclonal Anti-Rubella virus core protein IgG, aff pure
RUBL17-M	Monoclonal Anti-Rubella virus structural glycoprotein E1 IgG, aff pure

Instruction Manual No. M-510-100-HRG

Rubella IgG

ELISA KIT Cat. # 510-100-HRG

For the quantitative determination of IgG antibodies against Rubella Virus in Human Serum or Plasma

For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Rubella IgG ELISA KIT Cat. # 510-100-HRG (96 tests)

Kit Components (96 tests)	
Rubella IgG antigen coated strip plate, (8x12 strip or 96 wells) # 510-101	1 plate
Rubella IgG Std A (0 IU/ml) **Negative Control**, 2 mL #510-102A	1 vial
Rubella IgG Std B (10 IU/ml) **Cut-off control**, 2 mL #510-102B	1 vial
Rubella IgG Std C (50 IU/ml) **Weak Positive control** 2 mL #510-102C	1 vial
Rubella IgG Std D (200 IU/ml) **Positive control**, 2 mL #510-102D	1 vial
Rubella IgG Std E (500 IU/ml) **High Positive control** , 2 mL #510-102E	1 vial
All controls contain 0.02 % methylisothiazolone and 0.02 % bromonitrodioxane as preservative	
Anti-Human IgG-HRP Conjugate, (15 ml) #530-103	1 bottle
Diluent Buffer, 60 ml #510-100SD	1 bottle
Wash buffer (10X) 60 ml #510-100WB	1 bottle
TMB Substrate Solution, 15 ml #510-100-TMB	1 bottle
Stop Solution, 15 ml # 510-100ST	1 bottle
Complete Instruction Manual, M-510-100-HRG	1

Intended Use

ADI Rubella IgG Antibody ELISA Test Kit has been designed for the detection of IgG class antibodies against Rubella in human serum or plasma. This kits is for research use only (RUO).

Introduction

Rubella, commonly known as German measles, is a disease caused by the rubella (little red) virus. The name rubella is sometimes confused with rubeola, an alternative name for measles; the diseases are unrelated. Rubella is a common childhood infection usually with minimal systemic upset although transient arthropathy may occur in adults. The disease is caused by Rubella virus, a togavirus that is enveloped and has a single-stranded RNA genome. Rubella virus specific IgM antibodies are present in people recently infected by Rubella virus but these antibodies can persist for over a year and a positive test result needs to be interpreted with caution. Rubella infections are prevented by active immunization programs using live, disabled virus vaccines. Two live attenuated virus vaccines, RA 27/3 and Cendehill strains were effective in the prevention of adult disease.

MMR II vaccine is a mixture of three live attenuated viruses, administered via injection. The vaccine is sold by Merck as M-M-R II, GlaxoSmithKline Biologicals as Priorix, Serum Institute of India as Tresivac, and Sanofi Pasteur as Trimovax. The live viruses require animal or human cells as a host for production of more viruses. For example, in the case of mumps and measles viruses, the virus strains were grown in embryonated hens' eggs and chick embryo cell cultures. This produced strains of virus which were adapted for the hen's egg and less well-suited for human cells. These strains are therefore called attenuated strains. The Rubella component, Meruvax, is propagated using a human lung cell line (WI-38). The MMRV vaccine, a combined measles, mumps, rubella and varicella vaccine, has been proposed as a replacement for the MMR vaccine to simplify administration of the vaccines

Quality Control

The test results are only valid if the test has been performed following the instructions. All standards and kit controls must be found within the acceptable ranges as stated on the vials. The positive control must show at least double the OD of the cut-off standard. If criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls. In case of any deviation the following technical issues should be proven (reagents, protocol, equipments, etc).

PERFORMANCE CHARACTERISTICS

In an in-house study, apparently healthy subjects showed the following results:

		Interpretation		
Rubella	n	positive	equivocal	negative
IgG	**172**	**95.4 %**	**0 %**	**4.7 %**
IgM	**175**	**1.9 %**	**8.6 %**	**89.7 %**

It is recommended that each laboratory establishes its own range of normal values.

Precision:

Intra-Assay		
Sample	**Mean (IU/ml)**	**CV (%)**
1	8.6 -161	4.3 – 7.2

Inter-Assay		
Sample	**Mean (u/ml0**	**CV (%)**
1	8.2- 174	2.6 -17.0
Inter-lot	9.8- 413	5.3 -23.2

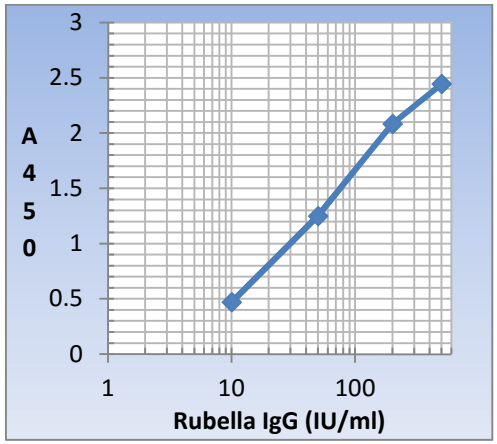
Analytical Sensitivity: 0.29 IU/ml
Linearity: Range (IU/ml): 58-227
Recovery:
Clinical Specificity: 100 %
Clinical sensitivity: 100 %

References: Altintas DU (1996) Med. Clin. 106, 647-648; Bayas JM (1996), Med. Clin. 106, 561-564; Chiu HH (1997) J. Med. Virol. 51, 32-35; DeSoouza VA (1997) J. Med. Virol. 52, 275-279; Duvdevani P (1996) Clin. Diagn. Virol. 7, 1-6; Narita M (1997) Clin. Diagn. Virol. 8, 233-239; Garces P (1995) Aten. Primaria 15, 235-237; Vardas E (1997) S. Afr. Med. J. 87, 1709

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450nm}	Calculated Conc. (IU/ml)
A1, A2	**Std. A** (0 IU/ml)	0.022	
B1, B2	**Std. B** (10 IU/ml)	0.471	
C1, C2	**Std. C** (50 IU/ml)	1.252	
D1, D2	**Std. D** (200 IU/ml)	2.086	
E1, E2	**Std. E** (500 IU/ml)	2.446	
G1, G2	**Sample 1**		IU/ml

NOTE: These data are for **demonstration purpose only**. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



3-ADI-graph
A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS:

The evaluation of the test can be performed either qualitatively or quantitatively.
Qualitative Evaluation The Cut-off value is given by the optical density (OD) of the Standard B (Cut-off standard). The Cut-off index (COI) is calculated from the mean optical densities of the sample and Cut-off value. Samples with higher ODs are positive, samples with lower ODs are negative. If the optical density of the sample is within a range of 20% around the Cut-off value (grey zone), the sample has to be considered as borderline. In such a case the repetition of the test with the same serum or with a new sample of the same patient, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run. For quantification, the Cut-off index (COI) of the samples can be as follows:

$$COI = \frac{OD\ Sample}{OD\ Standard\ B}$$

PRINCIPLE OF THE TEST

Alpha Diagnostic's Rubella IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Rubella antigens are bound on the surface of the microtiter strips. Patient serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Rubella antigen takes place. After an incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use antihuman-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated at room temp, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of the rubella IgG antibodies is directly proportional to the intensity of the color.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5µl, 100µl, 500µl) and multichannel pipet with disposable plastic tips. distilled water, reagent troughs, Orbital shaker, plate washer (recommended) and ELISA plate Reader (450nm).

PRECAUTIONS

Only for in-vitro use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed. All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken. Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly. All reagents have to be brought to room temperature (18 to 25 °C) before performing the test. Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided. It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions. When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time. In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used. No reagents from different kit lots have to be used, they should not be mixed among one another. All reagents have to be used within the expiry period. In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation. The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute the wash buffer with water (1:10)**.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. **All samples should be diluted 1:101 (5 ul samples in 500 ul sample diluent)**. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.

1. Label or mark the microtiter well strips to be used on the plate.

2. Dispense **100 ul** diluent in 1 well to be used as blank. Pipet **100 ul of** Prediluted controls, and samples (diluted 1:21) into appropriate wells in *duplicate*. See worksheet of a typical set-up on page 5. Cover the plate, mix gently for 5-seconds and **incubate at room temp for 60 min**.

3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 3 times** with 300 ul of 1X wash buffer. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Add **100 ul anti-IgG-HRP conjugate** to all wells. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp (25-28oC).

5. Wash the wells 3 times as in step 3.

6. Add **100 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and incubate for **20 minutes** at room temp. Blue color develops in positive controls and samples.

7. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 5-10 seconds to have uniform color distribution (blue color turns yellow).

8. Measure the **absorbance at 450 nm** using an ELISA reader within 60 min.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Do not touch the bottom of the wells.

Quantitative Evaluation:

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log. For the calculation of the standard curve, apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read directly from the standard curve. The Standards of his assay have been adjusted to WHO-Standard RUBI-1-94 (1st Intl. Standard).

The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Interpretation of results:

Method	Range	Interpretation
Quantitative (Standard curve)	< 8 IU/mL	negative
	8 – 12 IU/mL	equivocal
	> 12 IU/mL	positive
Qualitative (Cut-off Index, COI)	< 0.8	negative
	0.8 – 1.2	equivocal
	> 1.2	positive

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

Expected Values: An in-house study of normal human random samples showed the following results.

Rubella	n	Interpretation		
		positive	equivocal	negative
IgG	172	95.4 %	0	4.7 %
IgM	175	1.9 %	8.6 %	89.7 %

It is recommended that each laboratory establishes its own range of normal values.

LIMITATIONS OF THE PROCEDURE:

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details. For cross-reactivities, see PERFORMANCE. Azide and thimerosal at concentrations > 0.1 % interferes in this assay and may lead to false results. The following blood components do not have a significant effect (+/- 20% of expected) on the test results up to the below stated concentrations:

Hemoglobin	8.0 mg/mL
Bilirubin	0.3 mg/mL
Triglyceride	5.0 mg/mL