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Symbols Key

	Manufactured by
CE	CE Mark
EC REP	Authorized Representative
IVD	*In Vitro* Diagnostic Medical Device
REF	Catalog Number
	Consult Instructions for Use
LOT	Batch Code
EXP YYYY-MM-DD	"Use By" date in year-month-day format
2°C (35°F) Min / 30°C (86°F) Max	Temperature Limitation
Σ n	Contains sufficient for <n> tests
	Do not reuse
CONT	Contents
DEV	Test Device
SOL	Developer Solution
IFU	Instructions for Use
TEST RUB	Rubella Test

Patent No.: 5,559,041

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P-5203-E

BioSign® Rubella

Immunochromatographic assay for the qualitative detection of anti-Rubella Antibody (IgG) in human serum or plasma. An aid in the determination of immune status or for the confirmation of recent rubella infection.

For *In Vitro* Diagnostic Use

CLIA Complexity: Non-waived
CDC Analyte Identifier Code: 5510
CDC Test System Identifier Code: 49121

Catalog No.:	BSP-171-35	35 Test Kit
	BSP-171-10	10 Test Kit

Summary and Explanation

BioSign® Rubella is an immunochromatographic assay method which qualitatively detects anti-rubella IgG in human serum or plasma specimens. This test is intended for use as an indicator of immune status or for use with paired acute and convalescent specimens for determination of seroconversion as an aid in the diagnosis of recent rubella infection.

Rubella (German measles) is a benign, self-limiting disease, usually of childhood, which is characterized by mild upper respiratory symptoms, suboccipital lymphadenopathy, and an erythematous rash. Mild complications of arthralgias and arthritis may occur after the disappearance of rash in young adults.[1] Over the past 20 years, the administration of an attenuated rubella virus to prime target populations susceptible to the disease has markedly reduced the natural incidence of rubella infection.[2] At the present time, the prime indication for laboratory diagnosis of rubella resides in the potential risk of this disease to the fetuses of women in the early stages of pregnancy.[3] If contracted during the first trimester of pregnancy, the virus may produce a severe infection in the fetus resulting in multiple abnormalities referred to as congenital rubella syndrome. Additional consequences of rubella infection may include spontaneous abortion of the fetus or still birth.[4]

It is recommended that women of childbearing age be assessed by antibody analysis for susceptibility to rubella; those found susceptible should be vaccinated with due regard taken for the potential dangers of vaccination during pregnancy.[1,5]

Principle

BioSign® Rubella uses indirect solid-phase immunochromatographic assay technology for the qualitative detection of rubella antibodies (IgG class) in human serum or plasma. In the test procedure, 10 µL of serum or plasma specimen is added to the Specimen Well located directly below the Test Line Region. If any rubella antibody is present in the specimen, it will be captured by the rubella antigen band impregnated in the test membrane. The Developer Solution is then added to the large Developer Solution Well.

As the specimen and Developer Solution move by capillary action to the antigen band, the solution mobilizes the dye conjugated to human IgG antibodies. Visualization of the antigen band in the Test Line Region will occur only when the antibody-dye conjugate binds to the rubella IgG antibody which has been bound to the rubella antigen. As the antibody-dye conjugate continues to move along the test membrane, it will bind to another band located in the Control Line Region to generate a colored band regardless of the presence of rubella antibodies in the specimen. Therefore, the presence of two colored bands, one in the Test Line Region and the other in the Control Line Region, indicates a positive result, while the presence of only one colored band in the Control Line Region indicates a negative result.

Materials Provided

Store positive and negative controls at 2°- 8°C. Do Not Freeze. For *In Vitro* diagnostic use.

- Each test kit contains enough reagents and materials for 35 tests
- **BioSign® Rubella** test devices: containing a membrane strip coated with inactive rubella antigen and a pad impregnated with monoclonal anti-human IgG dye conjugate in a protein matrix containing 0.1% sodium azide
- Developer solution: Phosphate saline buffer, contains 0.1% sodium azide
- Positive control: Diluted serum (human), contains 0.1% sodium azide
- Negative control: Diluted serum (human), contains 0.1% sodium azide
- **BioSign® Rubella** package insert

Materials required but not provided:

- Vacutainer tubes for either serum or plasma procedure
- Centrifuge capable of 1500 x g*
- Micropipet to deliver a 10 µL volume

Precautions

Warning: The reagents in this kit contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large amount of water to prevent azide buildup.

- Do not interchange materials from different kit lots or use beyond the expiration date. Each kit is tested by Quality Control to function as a unit to assure proper sensitivity and maximum accuracy.
- Use **BioSign® Rubella** only in accordance with instructions supplied with the kit.
- All patient samples should be handled as if they are capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Each donor unit used in the preparation of the positive and negative controls was tested by an FDA approved method for the presence of the antibody to HIV and hepatitis B surface antigen and found to be negative. However, all materials should be handled as if capable of transmitting disease.

Specimen Collection and Preparation

Use serum or plasma obtained from blood collected aseptically by venipuncture into a clean tube. If serum or plasma filter isolates are used, follow the manufacturer's instructions.

For serum, no anticoagulant should be used. Blood should be allowed to clot at room temperature (18°-24°C) and then centrifuged at 1500 x g* for ten minutes at room temperature. The serum should be separated as soon as possible and can be tested immediately.

For plasma, collect the whole blood sample into a tube containing anticoagulant such as sodium citrate, heparin or EDTA.

Remove the serum or plasma from the clot or red cells as soon as possible to avoid hemolysis. Only When possible, clear, non-hemolyzed specimens should be used. Mildly hemolyzed samples do not affect the test result, but will create an undesirable reddish background in the reading window. Specimens containing any particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to testing.

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g* = 1.118x 10-5 x (rpm)2 or rpm = $\sqrt{g/R}$

R = centrifuge arm-sample holder length, cm (angular radius of the centrifuge head). Consult centrifuge manufacturer for details.

Specimen Storage

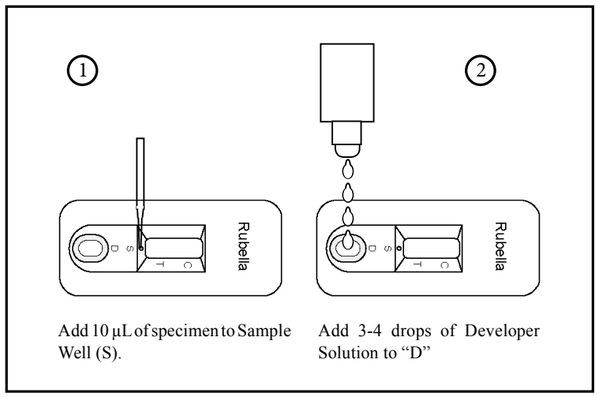
Refrigerate all specimens at 2°- 8°C until ready for testing. If specimens will not be tested within 48 hours of collection, they should be stored at -20°C or below. Specimens should not be repeatedly frozen and thawed. If specimens are to be mailed, they should be packed in appropriate shipping containers as currently described by the carrier services for handling of potentially infectious materials.

Warning: Specimens are potentially infectious; handle with appropriate precautions.

Procedure

Procedural notes

- The test protocol must be followed in order to achieve optimal test reactivity with specimens. Follow the assay procedure and always perform the test under carefully controlled conditions.
- Allow **BioSign® Rubella** test devices, reagents and specimens to warm to room temperature (21°- 30°C) before testing.
- The **BioSign® Rubella** test device should remain in the sealed pouch prior to testing.
- To avoid cross-contamination, use a new disposable micropipet tip for each specimen.
- To avoid contamination, do not touch the tip of the Developer Solution dropper bottle to skin or to the **BioSign® Rubella** test device.
- Use accepted microbiological practices for proper disinfection of potentially infectious test materials and contaminated equipment prior to disposal.
- After testing, dispose of **BioSign® Rubella** test devices, micropipet tips and specimens in approved biohazard containers.



Add 10 µL of specimen to Sample Well (S).

Add 3-4 drops of Developer Solution to "D"

Test Procedure

1. Label the device with the patient's name or control number.
2. Dispense 10 µL of specimen using a micropipet. Holding the micropipette vertically, allow the tip of the micropipet to touch lightly to the membrane under the Sample Well (S) and then release the contents by pressing the micropipet lever. (See image 1 above.)

   Caution: Do not add sample with the micropipet tip tilted toward the direction of the Developer Solution Well (D).
3. Holding the bottle of Developer Solution in a vertical position above the Developer Solution Well (D), dispense 3 to 4 drops into the well. (See image 2 above.)

   NOTE: Developer Solution should be added after specimen migration along the test membrane has been detected.
4. Read test results at 15 minutes. While some positive results may appear in as little as 6 minutes, waiting 15 minutes is required to report a negative result. Results are stable for up to 30 minutes after adding the Developer Solution.

Interpretation of Results

Positive:

Two pink-purple colored bands – one in the Test Line Region (T) and one in the Control Line Region (C) – indicate that rubella IgG antibodies have been detected.

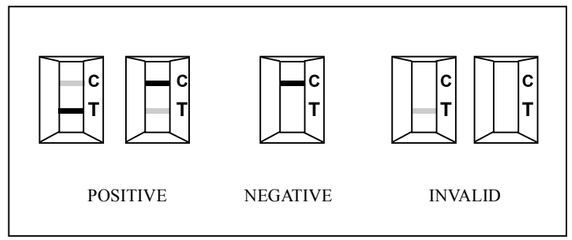
NOTE: A positive test result can be read as soon as distinct pink-purple colored bands appear in the Test Line Region (T) and Control Line Region (C). Any shade of pink-purple colored band in the Test Line Region should be reported as a positive result. The intensity of the colored band in the Test Line Region (T) may be different than the intensity of the band in the Control Line Region (C).

Negative:

One pink-purple colored band in the Control Line Region (C) with no distinct colored band in the Test Line Region (T) indicates that rubella IgG antibodies have not been detected.

Invalid:

A distinctive colored band should always appear in the Control Line Region (C) if the test is working properly. If no band forms in the Control Line Region (C), the test is invalid.



User Quality Control

Internal Quality Control

When the test has performed correctly and the device is working properly, a distinct colored line will always appear in the Control Line Region. The colored line in the Control Line Region is considered an internal positive procedural control. If the line does not appear, a new device should be tested. If the problem persists, contact PBM's Technical Service.

When the test has been performed correctly and the device is working properly, the background in the Result Window will clear, providing a distinct test result. This clearing background in the Result Window is considered an internal negative procedural control.

External Quality Control

A positive and negative external control provided in the kit must be tested with each new kit, lot or shipment and when required by your laboratory's QC procedure. If the controls do not perform as expected or the colored control band does not appear, contact PBM's Technical Services.

Limitations of the Procedure

- As is the case with any diagnostic procedure, the results obtained by this kit yield data which must be used only as adjunct to other information available to the physician.
- Specimens taken very early during the acute phase of infection with rubella virus may contain only IgM antibodies[6] and, therefore, may be negative by this procedure. **BioSign® Rubella** is a qualitative test for the detection of IgG antibodies against rubella virus.
- The amount of antibody necessary for an individual to be immune from rubella infection has not been firmly established.[7] However, a person with a weak positive result who is a candidate for vaccination may be retested using a second technique for a quantitative result.
- Appropriate timed and paired specimens may be used to determine recent infection.[8] Significant changes in the intensity of the test band may occur during the timed period. However, it may be useful in difficult cases to use a second technique such as hemagglutination inhibition test or other quantitative assay for IgM confirmation.
- Recent infections should be confirmed by an IgM test.
- Cross reactivity of the test system for antibodies to cytomegalovirus, herpes simplex virus, rubeola virus, and influenza virus have not been established.

Expected Values

Immune Status

The presence of rubella virus antibody (IgG class) demonstrates previous exposure to the rubella virus. Prevalence studies on the seroepidemiology of rubella indicate that in most countries 80-90% of the adult population has detectable antibodies to rubella.[9] The high prevalence rate is probably due to an early vaccination and exposure to rubella virus. Antibody titers of eight or greater by the hemagglutination- inhibition (HAI) assay indicate past rubella infection as immunity to primary infection.[11] **BioSign® Rubella** is capable of detecting antibody titers of eight or greater as determined by the HAI assay.

BioSign® Rubella detects antibodies at a level greater than or equal to 10 IU/mL, the level recommended by the Clinical and Laboratory Standards Institute (CLSI) to indicate immunity.

Recent or Active Infection

BioSign® Rubella can be used with paired acute and convalescent specimens collected at appropriate intervals for determination of seroconversion as an aid in the diagnosis of recent rubella infections. Timing of specimen collection is critical. Results should be confirmed by a quantitative assay for IgM. The seroconversion characteristic of recent or active infection may not be seen if the first (acute phase) specimen is taken too late or the second (convalescent phase) specimen is taken too early. The acute phase specimen should be collected as early as possible after the time of exposure or within seven days after the onset of symptoms. The convalescent phase specimen should be taken at least 14 days after the first specimen but not earlier than 10 days after the onset of symptoms.[10]

If no clinical symptoms occur, collect the specimen at least 30 days after exposure. Both the acute and convalescent specimens should be tested simultaneously. In the case of seroconversion, significant changes in the test band intensity or in the timing of the test band appearance will occur when using **BioSign® Rubella**.

Performance Characteristics

A total of 612 blind clinical specimens consisting of 479 serum samples and 133 plasma samples were assayed for IgG antibody to rubella virus with **BioSign® Rubella** and a commercially available latex agglutination test. The agreement between the two test systems was 99% (606/612). **BioSign® Rubella** demonstrated a relative sensitivity of 98.9% (437/442) and relative specificity of 99.4% (169/170) when compared with the reference test (see Table 1).

Table 1. BioSign® Rubella vs. Comparative Method

Serum		Comparative Method		
		+	–	Total
BioSign® Rubella	+	437	1	438
	-	5	169	174
	Total	442	170	612

These data demonstrate the excellent correlation between **BioSign® Rubella** and a commercially available latex agglutination test.

Of the 133 plasma samples, 22 were identified as positive by **BioSign® Rubella** and all 22 samples were confirmed as positive it is these assay results. The remaining 111 samples were confirmed negative by the reference assay and 110 samples tested negative by **BioSign® Rubella** with one false positive result. The following table summarizes the results of the study with plasma samples (see Table 2).

Table 2. Reference Results

	Anti-Coagulant		Positive	Negative
BioSign® Rubella	Sodium Citrate	Positive	12	0
		Negative	0	88
	Heparin	Positive	4	1
		Negative	0	9
	EDTA	Positive	6	0
		Negative	0	13
	Total		22	111

Sensitivity

BioSign® Rubella has a relative sensitivity of 10 IU/mL or greater of 2nd International Preparation. This was determined by testing 1/2 dilution of Low-Titer Anti-Rubella Human Reference Sera CDC Biological Standard (Cat# 85-0120, Lot # IS2153, 21 IU/mL) and by testing 1/3 dilution of CAP Reference Standard Level 1 (Cat# RM003, Lot# 1967107A, 32.7 ±1.6 IU/mL). Further, the ability of the test to detect rubella antibody in a rubella-positive serum was established by detecting with 99% accuracy over 200 positive clinical samples including 55 low positive samples in the range of 10 to 20 IU/mL.

Specificity

The accuracy of **BioSign® Rubella** in discerning a rubella-negative serum, with an antibody level less than 10 IU/mL, from a rubella-positive serum was established to be 99% from the test of 170 samples.

Proficiency and Reproducibility Evaluation

An intra-laboratory reproducibility study was performed using three lots of devices in triplicate on three days for a total of 270 tests. Five positive (20 IU/mL) and five negative samples were used for testing of the three lots for a total of 90 tests each day. The results obtained agreed 100% with expected results.

An inter-laboratory reproducibility study for the test proficiency evaluation was performed at three locations. At each location, five positive (20 IU/mL) and five negative samples were tested in duplicate for a total of 60 tests conducted. The results obtained at each site agreed 100% with expected results.

Within-run Reproducibility Testing

Twenty replicate tests of low positive samples (15 IU/mL) and twenty replicate tests of non-reactive sera (below 10 IU/mL) showed 100% reproducibility of the test. To confirm the intended use in immune status determination, 55 rubella-reactive samples with a titer in the range of 10 to 20 IU/mL and 65 non-reactive samples were tested. The results showed 100% confirmation of the expected results.

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