Foresight®

HBsAg EIA Test Kit Package Insert

REF I231-1021 | English

An enzyme immunoassay (EIA) for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in human serum or plasma.
For professional in vitro diagnostic use only.

INTENDED USE

The HBsAg EIA Test Kit is a one step enzyme immunoassay for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in human serum or plasma. It is intended for screening and as an aid in the diagnosis of possible Hepatitis B infection.

SUMMARY

HBsAg is one of the earliest markers that appear in the blood following infection with Hepatitis B virus (HBV).[1] This infection of the liver is transmitted through sexual contact, blood borne exposure, transmission from mother to child during delivery, sharing of objects that pierce the skin, child-to-child and household contact. The four major HBsAg subtypes include adw, adr, ayw, and ayr, all sharing the common determinant 'a'. The HBV infection causes a wide variety of liver damage such as acute self-limiting infection, fulminating hepatitis, chronic hepatitis with progression to cirrhosis and liver failure, and asymptomatic chronic carrier state. In HBV infected people, the virus persists for the rest of their lives and can be passed on to others. Therefore, Hepatitis B has become a global public health problem. Infection with HBV results in the appearance of a number of serological markers and one of the first of such markers is Hepatitis B Surface Antigen (HBsAg). HBsAg appears 1-10 weeks after exposure and before biochemical evidence of liver disease or jaundice.[2,3] Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, HBsAg persists for 6-12 months with no seroconversion to the corresponding antibodies. Therefore, screening for HBsAg is highly recommended for all donors, pregnant women and people in high-risk groups.
The HBsAg EIA Test Kit is a third generation immunoassay for the qualitative detection of the presence of Hepatitis B Surface Antigen in serum or plasma specimen. The test utilizes monoclonal antibodies to selectively detect various subtypes of HBsAg in serum or plasma.

PRINCIPLE

The HBsAg EIA Test Kit is a solid phase qualitative enzyme immunoassay based on a sandwich principle for the detection of HBsAg in human serum or plasma. The microwell plate is coated with monoclonal antibodies specific to various subtypes of HBsAg. During testing, the specimen and the enzyme-conjugated HBsAg antibodies are added to the antibody coated microwell plate and then incubated. If the specimen contains HBsAg, it will bind to the antibodies coated on the microwell plate and simultaneously bind to the conjugate to form immobilized antibody-HBsAg-conjugate complexes. If the specimen does not contain HBsAg, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color, indicating the amount of HBsAg present in the specimen. Sulfuric acid solution is added to the microwell plate to stop the reaction which produces a color change from blue to yellow. The color intensity, which corresponds to the amount of HBsAg present in the specimen, is measured with a microplate reader at 450/630-700 nm or 450 nm.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Substrate and Controls. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0.5M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The HBsAg EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity 96 wells/kit	Quantity 480 wells/kit
	HBsAg Microwell Plate	Microwell plate coated with Anti-HBsAg	1 plate (96 wells/ plate)	5 plates (96 wells/ plate)
1	HBsAg Conjugate	Anti-HBsAg bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300	1 x 40 mL	5 x 40 mL
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 mL	5 x 8 mL
5	Stop Solution	0.5M Sulfuric acid	1 x 8 mL	5 x 8 mL
6	HBsAg Negative Control	Normal serum non-reactive for HBsAg, HCV, HIV-1, and HIV-2; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
7	HBsAg Positive Control	Inactivated serum containing HBsAg and negative for HCV, HIV-1, and HIV-2; Preservative: 0.1% ProClin™ 300	1 x 1 mL	5 x 1 mL
	Plate Sealers		2	10
	Package Insert		1	1

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Automated processor (optional)

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the controls so that well A1 is the Blank well. From well A1, arrange the controls in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1000 mL for 96 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 15-30°C. **Note:** If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. • Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 • Remove and store unused strips at 2-8°C
0	• Leave A1 as Blank well.	• Leave A1 as Blank well
1	• Add 100 µL of Negative Control in wells B1 and C1. (Blue Reagent) • Add 100 µL of Positive Control in wells D1 and E1. (Red Reagent) • Add 100 µL of specimen to assigned wells starting at F1.	• B1 and C1: Add 100 µL Negative Control • D1 and E1: Add 100 µL Positive Control • Starting F1: Add 100 µL specimen
2	• Add 50 µL of Conjugate to each well except for the Blank well. (Red Reagent) **NOTE:** To avoid contamination, drop the conjugate on the bottom of the well vertically; be careful not to touch the inner surface of the well with the pipette tip.	• Add 50 µL of Conjugate to each well except for the Blank well

3	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds.
	• Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator using one of the following procedures:
	  • Standard Procedure: Incubate at 37°C ± 2°C for 60 minutes ± 2 minutes.
	  • Enhanced Procedure: Incubate at 37°C ± 2°C for 120 minutes ± 2 minutes.
		• Mix gently
		• Cover the microwell plate with the Plate Sealer and incubate using one of the following procedures:
		  • Standard Procedure: Incubate at 37°C for 60 min
		  • Enhanced Procedure: Incubate at 37°C for 120 min

4	• Remove the Plate Sealer. • Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid. • Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried **NOTE:** Incomplete wash can cause false positive. Ensure no less than 5 washing cycles with dispensing of 350µL/well. Ensure there's no overflow and no liquid is left in the well. Tap out any residual wash fluid on to absorbent paper after washing.	• Remove the Plate Sealer • Wash each well 5 times with 350 µL of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue

5	• Add 50 µL of Substrate A to each well. (Clear Reagent) • Add 50 µL of Substrate B to each well. (Clear Reagent) • Substrate A and Substrate B can also be mixed together as a Working Substrate Solution before use. Add a volume of Substrate A to an equal volume of Substrate B in a clean glass or plastic vessel; mix well. Add 100 µL Working Substrate Solution to each well. **Note:** The Working Substrate Solution should be used within 30 minutes. • Then a blue color should develop in wells containing Positive specimens.	• Add 50 µL of Substrate A to each well • Add 50 µL of Substrate B to each well • Or add 100 µL Working Substrate Solution to each well.
6	• Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator using one of the following procedures: • Standard Procedure: Incubate at 37°C ± 2°C for 10 minutes ± 1 minute. • Enhanced Procedure: Incubate at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Mix then cover microwell plate with Plate Sealer and incubate using one of the following procedures: • Standard Procedure: Incubate at 37°C for 10 min • Enhanced Procedure: Incubate at 37°C for 30 min
7	• Remove the Plate Sealer. • Add 50 µL of Stop Solution to each well. (Clear Reagent) Then a yellow color should develop in wells containing Positive specimens.	• Remove the Plate Sealer • Add 50 µL of Stop Solution to each well
8	• Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.	• Read at 450/630-700 nm within 30 min

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Negative Control and Positive Control by referring to the table below.

Example of Negative Control Calculation

Item	Absorbance
Negative Control: Well B1	0.023
Negative Control: Well C1	0.021
Total Absorbance of Negative Control	0.023 + 0.021 = 0.044
Mean Absorbance of Negative Control	0.044/2 = 0.022
Blank Absorbance: Well A1	0.002
NCx: Mean Absorbance of Negative Control – Blank Absorbance	0.022 - 0.002 = 0.020

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Negative Control	Mean Absorbance after subtraction of Blank Absorbance should be < 0.100
Positive Control	Mean Absorbance after subtraction of Blank Absorbance should be > 1.000

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

3. Calculate the Cut-Off Value using the following formula if the test results are valid.

Example of Cut-Off Value Calculation

Item	Absorbance
NCx	0.020
Cut-Off Value: NCx + 0.070	0.020 + 0.070 = 0.090

INTERPRETATION OF RESULTS

NON-REACTIVE: Specimens with absorbance less than the Cut-Off Value are non-reactive for HBsAg and may be considered negative.

REACTIVE:* Specimens with absorbance greater than or equal to the Cut-Off Value are considered initially reactive for HBsAg. The specimen should be retested in duplicate before final interpretation. Specimens that are reactive in at least one of the re-test are presumed to be repeatedly reactive and should be confirmed using other HBV markers or confirmatory testing. Specimens that are non-reactive on both retests should be considered non-reactive.

***NOTE:** Specimens with values within ±10% of the Cut-Off Value should be retested in duplicates for final interpretation.

LIMITATIONS

1. The HBsAg EIA Test Kit is used for the detection of HBsAg in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A non-reactive test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results. Mutated HBsAg may not be detected by the test.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that non-repeatable reactive results may occur due to inadequate washing. The results may be affected due to procedural or instrument error.
4. False positive results may occur due to high titers of Heterophilic Anti Mouse Antibodies (HAMA). Erroneous result may also be due to fibrin particles and microbial contamination.
5. False negative results may occur if the quantity of HBsAg present in the specimen is lower than the analytical sensitivity of the test, or if HBsAg is not present during the stage of disease when the specimen was collected.
6. The Positive Control in the test kit is not to be used to quantify assay sensitivity. The Positive Control is used to verify that the test kit components are capable of detecting a reactive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

The analytical sensitivity of the HBsAg EIA Test Kit has been determined using reference HBsAg standards, ad and ay subtypes. The analytical sensitivity is 0.2 IU/mL using the standard procedure and 0.1 IU/mL using the enhanced procedure, which were all confirmed using the WHO NISBC International Standard with code number 01/476-011-WIL for HBsAg.

Clinical Sensitivity and Specificity

The HBsAg EIA Test Kit has correctly identified specimens of a seroconversion panel and has been compared with a leading commercial HBsAg EIA test using clinical specimens. The results show that the clinical sensitivity of the HBsAg EIA Test Kit is >99.9%, and the clinical specificity is 99.9%.

HBsAg EIA vs. Other EIA

Method		Other EIA		Total Results
	Results	Positive	Negative	
HBsAg EIA	Positive	562	3	565
	Negative	0	5,234	5,234
Total Results		562	5,237	5,799

Clinical Sensitivity: >99.9% (99.4-100.0%)* Clinical Specificity: 99.9% (99.8-100.0%)*
Overall Agreement: 99.9% (99.9-100.0%)* *95% Confidence Interval

Reproducibility

Intra-Assay: Within-run precision has been determined by using 10 replicates of three specimens: a low positive, a medium positive, and a high positive.

Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, a medium positive, and a high positive. Three different lots of the HBsAg EIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance /Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	1.467	0.008	6.061	1.367	0.011	8.943
2	12.622	0.060	5.282	13.378	0.091	7.558
3	26.722	0.096	3.992	25.467	0.225	9.817

BIBLIOGRAPHY

1. Blumberg, B.S. The Discovery of Australian Antigen and its Relation to Viral Hepatitis. Vitro. 1971;7:223.
2. Krugman, S. Glies J.P. *Viral Hepatitis, Type B (MS-2-Strain). Further Observations on Natural History and Prevention*. New England Journal of Medicine. 288, 755.
3. Krugman, S. Overby L.R, et al. *Viral Hepatitis Type B Studies On Natural History and Prevention Re-examined*. New England Journal of Medicine. 300, 101.

Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by		
2°C–8°C	Store between 2-8°C	LOT	Lot Number	REF	Catalog #
HBsAg	HBsAg	Substrate A	Substrate A		
Wash Buffer 25x	Wash Buffer (25x)	Conjugate	Conjugate	Substrate B	Substrate B
Control −	Negative Control	Plate Sealer	Plate Sealer	Control +	Positive Control
Microwell Plate	Microwell Plate	Stop Solution	Stop Solution	Package Insert	Package Insert

ACON®

ACON Laboratories, Inc.
10125 Mesa Rim Road,
San Diego, CA 92121, USA

Foresight®

HCV Antibody EIA Test Kit
Package Insert

REF 1231-1031	English

An enzyme immunoassay (EIA) for the qualitative detection of IgG antibodies to Hepatitis C Virus (HCV) in human serum or plasma.
For professional in vitro diagnostic use only.

INTENDED USE

The HCV Antibody EIA Test Kit is a qualitative enzyme immunoassay for the detection of IgG antibodies to Hepatitis C Virus (HCV) in human serum or plasma. It is intended for screening and as an aid in the diagnosis of possible Hepatitis C infection.

SUMMARY

Hepatitis C Virus is a small, enveloped, positive-sense, single-stranded RNA Virus. HCV is now known to be the major cause of parenterally transmitted non-A, non-B hepatitis. HCV infection causes a wide variety of chronic liver disease, cirrhosis and liver cancer. The main route of transmission of the virus is via transfusion of blood and blood products, organ transplantation, and sharing contaminated needles and syringes. Antibodies to HCV is found in over 80% of patients with well-documented non-A, non-B hepatitis. Cloning the viral genome has made it possible to develop serologic assays that use recombinant antigens.[1,2] Compared to the first generation HCV EIA tests using single recombinant antigen, new serologic tests incorporate recombinant protein and/or synthetic peptide antigens to avoid nonspecific cross-reactivity and to increase the sensitivity.[3,4]
The HCV Antibody EIA Test Kit is a third generation immunoassay for the qualitative detection of the presence of IgG antibodies to HCV in serum or plasma specimen. The test utilizes recombinant HCV antigens encoded by the genes for both structural (nucleocapsid) and non-structural proteins to selectively detect antibodies to HCV in serum or plasma.

PRINCIPLE

The HCV Antibody EIA Test Kit is a solid phase qualitative indirect simultaneous enzyme immunoassay for the detection of IgG antibodies to HCV in human serum or plasma. The microwell plate is coated with HCV recombinant antigens. During testing, the specimen diluent and the specimens are added to the antigen coated microwell plate and then incubated. If the specimens contain antibodies to HCV, it will bind to the antigens coated on the microplate to form immobilized antigen-HCV antibody complexes. If the specimens do not contain antibodies to HCV, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies are added to the microwell plate and incubated. The enzyme-conjugated anti-human IgG antibodies will bind to the immobilized antigen-HCV antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of HCV antibodies present in the specimen. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of HCV antibodies present in the specimen, is measured with a microplate reader at 450/630-700 nm or 450 nm.

PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate and Controls. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Avoid any contact of Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0.5M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

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- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The HCV Antibody EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS AND COMPONENTS

Materials Provided

No.	Reagent	Component Description	Quantity 96 wells/kit	Quantity 480 wells/kit
	HCV Microwell Plate	Microwell plate coated with recombinant HCV antigens	1 plate (96 wells/plate)	5 plates (96 wells/plate)
1	HCV Conjugate	Anti-human IgG antibody bound to peroxidase; Preservative: 0.1% ProClin™ 300	1 x 12 ml	5 x 12 ml
2	Concentrated Wash Buffer (25x)	Tris-HCl buffer containing 0.1% Tween 20 Preservative: 0.1% ProClin™ 300	1 x 50 ml	5 x 50 ml
2A	Specimen Diluent	Tris buffer Preservative; Preservative: 0.1% ProClin™ 300	1 x 12 ml	5 x 12 ml
3	Substrate A	Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300	1 x 8 ml	5 x 8 ml
4	Substrate B	Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300	1 x 8 ml	5 x 8 ml
5	Stop Solution	0.5M Sulfuric acid	1 x 8 ml	5 x 8 ml
6	HCV Negative Control	Normal serum non-reactive for HCV, HBsAg, HIV-1, and HIV-2; Preservative: 0.1% ProClin™ 300	1 x 0.4 ml	5 x 0.4 ml
7	HCV Positive Control	Inactivated serum containing antibodies to HCV and negative for HBsAg, HIV-1, and HIV-2; Preservative: 0.1% ProClin™ 300	1 x 0.4 ml	5 x 0.4 ml
	Plate sealers		3	15
	Package insert		1	1

Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µl/well
- Disposable gloves
- Automated processor (optional)
- Calibrated micropipettes with disposable tips capable of dispensing 10, 50, and 100 µl
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter

DIRECTIONS FOR USE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the controls so that well A1 is the Blank well. From well A1, arrange the controls in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

Step	Detailed Procedure	Simplified Procedure
	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 ml for 96 wells/plate testing. The Working Wash Buffer is stable for 2 weeks at 15-30°C. **Note:** If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve. • Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.	• Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25 • Remove and store unused strips at 2-8°C
0	• Leave A1 as Blank well.	• Leave A1 as Blank well
1	• Add 100 µl Specimen Diluent in respective wells including Negative Control, Positive Control, Blank and specimen wells. (Green Reagent)	• Add 100 µl Specimen Diluent
2	• Add 10 µl of Negative Control in wells B1 and C1. (Blue Reagent) • Add 10 µl of Positive Control in wells D1 and E1. (Red Reagent) • Add 10 µl of specimen to assigned wells starting at F1. Then a color change from green to blue will occur to verify that the specimen has been added.	• B1 and C1: Add 10 µl Negative Control • D1 and E1: Add 10 µl Positive Control • Starting F1: Add 10 µl specimen
3	• Mix gently by swirling the microwell plate on a flat bench for 30 seconds. • Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Mix gently • Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
4	• Remove the Plate Sealer. • Wash each well 5 times by filling each well with 350 µl of Working Wash Buffer, then remove the liquid. • Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried. Note: Improper washing may cause false positive results.	• Remove the Plate Sealer • Wash each well 5 times with 350 µl of Working Wash Buffer • Turn the microwell plate upside down on absorbent tissue
5	• Add 100 µl of Conjugate to each well except for the Blank well. (Red Reagent)	• Add 100 µl of Conjugate to each well except for the Blank well

6	• Cover with microwell plate with the Plate Sealer and incubate in a waterbath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.	• Cover the microwell plate with the Plate Sealer and incubate at 37°C for 30 min
7	• Repeat Step 4.	• Repeat Step 4
8	• Add 50 µl of Substrate A to each well. (Clear Reagent) • Add 50 µl of Substrate B to each well. (Clear Reagent) • Substrate A and Substrate B can also be mixed together as a Working Substrate Solution before use. Add a volume of Substrate A to an equal volume of Substrate B in a clean glass or plastic vessel; mix well. Add 100 µL Working Substrate Solution to each well. **NOTE:** The Working Substrate Solution should be used within 30 minutes. • Then a blue color should develop in wells containing Positive specimens.	• Add 50 µl of Substrate A to each well • Add 50 µl of Substrate B to each well • Or add 100 µL Working Substrate Solution to each well.
9	• Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.	• Mix then cover microwell plate with Plate Sealer and incubate at 37°C for 10 min
10	• Remove the Plate Sealer. • Add 50 µl of Stop Solution to each well. (Clear Reagent) Then a yellow color should develop in wells containing Positive specimens.	• Remove the Plate Sealer • Add 50 µl of Stop Solution to each well
11	• Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.	• Read at 450/630-700 nm within 30 min

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Negative Control and Positive Control by referring to the table below.

Example of Negative Control Calculation

Item	Absorbance
Negative Control: Well B1	0.014
Negative Control: Well C1	0.012
Total Absorbance of Negative Control	0.014 + 0.012 = 0.026
Mean Absorbance of Negative Control	0.026/2 = 0.013
Blank Absorbance: Well A1	0.006
NCx: Mean Absorbance of Negative Control – Blank Absorbance	0.013 – 0.006 = 0.007

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Negative Control	Mean Absorbance after subtraction of Blank Absorbance should be < 0.100
Positive Control	Mean Absorbance after subtraction of Blank Absorbance should be > 1.000

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

3. Calculate the Cut-Off Value using the following formula if the test results are valid.

Example of Cut-Off Value Calculation

Item	Absorbance
NCx	0.007
Cut-Off Value: NCx + 0.145	0.007 + 0.145 = 0.152

INTERPRETATION OF RESULTS

NON-REACTIVE: Specimens with absorbance less than the Cut-Off Value are non-reactive for antibodies to HCV and may be considered negative.

REACTIVE:* Specimens with absorbance greater than or equal to the Cut-Off Value are considered initially reactive for antibodies to HCV. The specimen should be retested in duplicate before final interpretation. Specimens that are reactive in at least one of the re-test are presumed to be repeatedly reactive and should be confirmed using confirmatory testing. Specimens that are non-reactive on both retests should be considered non-reactive.

***NOTE:** Specimens with values within ±10% of the Cut-Off Value should be retested in duplicates for final interpretation.

LIMITATIONS

1. The HCV Antibody EIA Test Kit is used for the detection of antibodies to HCV in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A non-reactive test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
3. As with other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. The results may be affected due to procedural or instrument error.
4. The Positive Control in the test kit is not to be used to quantify assay sensitivity. The Positive Control is used to verify that the test kit components are capable of detecting a reactive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity

The HCV Antibody EIA Test Kit has correctly identified specimens of a seroconversion panel and has been compared to a leading commercial EIA HCV test using clinical specimens. The results show that the clinical sensitivity of the HCV Antibody EIA Test Kit is >99.9%, and the clinical specificity is 99.8%.

HCV Antibody EIA vs. Other EIA

Method		Other EIA		Total Results
HCV Antibody EIA	Results	Positive	Negative	
	Positive	297	10	307
	Negative	0	5,897	5,897
Total Results		297	5,907	6,204

Clinical Sensitivity: >99.9% (97.0-100.0%)* Clinical Specificity: 99.8% (99.5-100.0%)*
Overall Agreement: 99.8% (99.6-100.0%)* *95% Confidence Interval

Reproducibility

Intra-Assay: Within-run precision has been determined by using 15 replicates of three specimens: a low positive, medium positive and a high positive.

Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, a medium positive and a high positive. Three different lots of the HCV Antibody EIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	Intra-Assay			Inter-Assay		
	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)	Mean Absorbance/ Cut-Off	Standard Deviation	Coefficient of Variation (%)
1	3.259	0.213	6.535	3.731	0.312	8.362
2	6.168	0.404	6.549	7.811	0.630	8.066
3	16.712	0.970	5.804	14.445	0.983	6.805

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2. Kuo, G., Q.L. Choo, H.J. Alter, and M. Houghton. *An Assay for Circulating Antibodies to a Major Etiologic Virus of Human Non-A, Non-B Hepatitis*. Science. 1989;244:362.
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Index of Symbols

	Consult instructions for use		Tests per kit		Manufacturer
IVD	For *in vitro* diagnostic use only		Use by	2°C 8°C	Store between 2-8°C
HCV	HCV	LOT	Lot Number	REF	Catalog #
Wash Buffer 25x	Wash Buffer (25x)	Substrat A	Substrate A	Substrat B	Substrate B
Control −	Negative Control	Conjugate	Conjugate	Control +	Positive Control
Microwell Plate	Microwell Plate	Stop Solution	Stop Solution	Package Insert	Package Insert
Specimen Diluent	Specimen Diluent	Plate Sealer	Plate Sealer		

***ACON* Laboratories, Inc.**
10125 Mesa Rim Road,
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Atlas Medical CE

ATLAS C-REACTIVE PROTEIN (CRP) LATEX KIT

For the qualitative and semi-quantitative measurement of C-reactive protein (CRP) in human serum.

IVD *For in -vitro diagnostic and professional use only*

2°C 8°C Store at 2-8°C

INTENDED USE

Atlas C-Reactive Protein (CRP) is used to measure the CRP in human serum qualitatively and semi- quantitatively.

INTRODUCTION

C-reactive protein (CRP), the classic acute-phase of human serum, is synthesized by hepatocytes. Normally, it is present only in trace amounts in serum, but it can increase as much as 1,000-fold in response to injury or infection. The clinical measurement of CRP in serum therefore appears to be a valuable screening test for organic disease and a sensitive index of disease activity in inflammatory, infective and ischemic conditions. MacLeod and Avery found that antibody produced against purified CRP provided a more sensitive test than the C-polysaccharide assay. Since that time a number of immunological assays have been devised to measure CRP such as capillary precipitation, double immunodiffusion and radical immunodiffusion.

The CRP reagent kit is based on the principle of the latex agglutination assay described by Singer and Plotz. The major advantage of this method is the rapid two (2) minute reaction time.

PRINCIPLE

The CRP reagent kit is based on an immunological reaction between CRP Antisera bound to biologically inert latex particles and CRP in the test specimen. When serum containing greater than 6 mg/L CRP is mixed with the latex reagent, visible agglutination occurs.

MATERIALS

MATERIALS PROVIDED

- CRP Latex Reagent:Latex particles coated with goat IgG anti-human CRP, pH 8.2 **MIX WELL BEFORE USE.**
- CRP Positive Control Serum: A stabilized pre-diluted human serum containing >20mg/L CRP.
- CRP Negative Control Serum: A stabilized pre-diluted animal serum.
- Glass Slides.
- Stirring Sticks.

MATERIALS REQUIRED BUT NOT PROVIDED

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL.
- Glycine Buffer (20x): add one part to nineteen parts of distilled water before use.

PRECAUTIONS

- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In Vitro diagnostic use.
- Positive and negative controls prepared using human serum found negative for hepatitis B surface antigen (HBsAg) by FDA required test; however, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper provided with the latex and hold perpendicularly when dispensing.
- Glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.

STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2 - 8°C). **DO NOT FREEZE.**
- The CRP latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.

SPECIMEN COLLECTION AND STORAGE

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8 °C and for 3 months at -20°C.
- For longer periods the sample must be frozen.
- As in all serological tests, hemolytic or contaminated serum must not be used.
- Do not use plasma.

PROCEDURE

A.QUALITATIVE TEST:

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 40 µL of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the CRP-latex reagent vigorously or on a vortex mixer before using and add one drop (40 µL) next to the samples to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

B.SEMI-QUANTITATIVE TEST:

1. Make serial two fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

QUALITY CONTROL

Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.

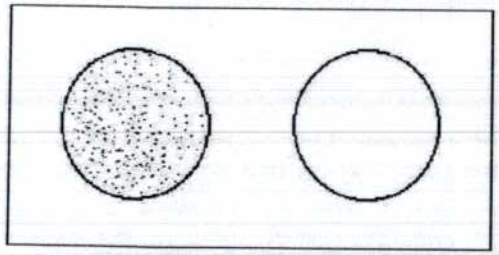
All result different from the negative control result, will be considered as a positive.

INTERPRETATION OF RESULTS

A.QUALITATIVE TEST:

A **negative** reaction is indicated by a uniform milky suspension with no agglutination as observed with the CRP Negative Control.

A **positive** reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the CRP Negative Control (Fig. 1).



Positive **Negative**

Figure 1

B. Semi-QUANTITATIVE TEST:

The approximate CRP concentration in the patient sample is calculated as follow:

6×CRP titer = ---- mg/L

INTERFERENCES

NONE INTERFERING SUBSTANCES:

- Hemoglobin (10g/dl)
- Bilirubin(20mg/dl)
- Lipemia(10g/dl)
- Other substances interfere, such as RF (100IU/ml).

NOTE

- High CRP concentration samples may give negative results .Retest the sample again using a drop of 20µl.
- The strength of agglutination is not indicative of the CRP concentration in the samples tested.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

LIMITATIONS

1. Reaction time is critical. If reaction time exceeds two (2) minutes, drying of the reaction mixture may cause false positive results.
2. Freezing the CRP Latex Reagent will result in spontaneous agglutination.
3. Intensity of agglutination is not necessarily indicative of relative CRP concentration; therefore, screening reactions should not be graded.
4. A false negative can be attributed to a prozone phenomenon (antigen excess). It is recommended, therefore, to check all negative sera by retesting at a 1:10 dilution with glycine buffer.

REFERENCE VALUES

Up to 6 mg/L. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

- **Sensitivity:** 6(5-10) mg/L
- **Prozone effect:** No prozone effect was detected up to 1600 mg/L
- **Diagnostic sensitivity:** 95.6 %.
- **Diagnostic specificity:** 96.2 %.

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PPI005A01
Rev H (06.06.2017)

REF	Catalogue Number		Store at
IVD	For In-Vitro Diagnostic use		Caution
Σ	Number of tests in the pack		Read product insert before use
LOT	Lot (batch) number		Manufacturer
	Fragile, handle with care		Expiry date
	Manufacturer fax number		Do not use if package is **damaged**
	Manufacturer telephone number		

Atlas Medical CE

ATLAS RHEUMATOID FACTOR (RF) LATEX KIT

latex slide test for the qualitative and semi-quantitative measurement of RF in human serum.

IVD *For In-Vitro diagnostic and professional use only*

2°C 8°C Store at 2-8°C

INTENDED USE

A latex slide test for the qualitative and semi-quantitative measurement of RF in human serum.

INTRODUCTION

Rheumatoid factors (RF) are antibodies directed against antigenic sites in the Fc fragment of human and animal IgG . Their frequent occurrence in rheumatoid arthritis makes them useful for diagnosis and monitoring of the disease.

One method used for rheumatoid factor detection is based on the ability of rheumatoid arthritis sera to agglutinate sensitized sheep red cells, as observed by Waaler and Rose· A more sensitive reagent consisting of biologically inert latex beads coated with human gamma globulin was later described by Singer and Plotz. The RF kit is based on the principle of the latex agglutination assay of Singer and Plotz .The major advantage of this method is rapid performance (2 minute reaction time) and lack of heterophile antibody interference.

PRINCIPLE

The RF reagent is based on an immunological reaction between human IgG bound to biologically inert latex particles and rheumatoid factors in the test specimen. When serum containing rheumatoid factors is mixed with the latex reagent, visible agglutination occurs.

MATERIALS

MATERIALS PROVIDED

- RF Latex Reagent: Latex particles coated with human gamma-globulin, pH, 8,2. Preservative. Contains N, N-dimethylformamide .
- RF Positive Control Serum: Human serum with a RF concentration > 30 IU/mL.Preservative.
- RF Negative Control Serum:Animal serum. Preservative.
- Reaction Slide
- Stirring sticks

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Test Tubes (for dilution)
- Serological pipettes (for sample addition and for dilution)
- Rotator (optional)
- Glycine Buffer (20x): add one part to nineteen parts of distilled water before use.

PRECAUTIONS

- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In Vitro diagnostic use.
- Positive and negative controls prepared using human serum found negative for hepatitis B surface antigen (HBsAg) by FDA required test; however, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.

STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2-8°C).
- Do not freeze.
- The RF latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.

SPECIMEN COLLECTION AND STORAGE

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- As in all serological tests, hemolytic or contaminated serum must not be used.
- Do not use PLASMA.

PROCEDURE

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50 µL of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the RF-latex reagent rigorously or on a vortex mixer before using and add one drop (50 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

Semi-quantitative method

1. Make serial two fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator. The presence of agglutination indicates a RF concentration equal or greater than 8 IU/mL (Note 1).
The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

CALCULATIONS

The approximate RF concentration in the patient sample is calculated as follows:

8 x RF Titer = IU/mL

INTERFERENCES

NON INTERFERING SUBSTANCES:

- Hemoglobin (10g/dl)
- Bilirubin(20mg/dl)
- Lipemia(10g/dl)

Other substances may interfere.

QUALITY CONTROL

1. RF Positive and Negative Control should be included in each test batch.
2. Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the RF Negative Control and agglutination with large aggregates is observed with the RF Positive Control.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity

8(6-16) IU/ml, under the described assay conditions.

PROZONE EFFECT

No prozone effect was detected up to 1500 IU/ml.

DIAGNOSTIC SENSITIVITY

100%.

DIAGNOSTIC SPECIFICITY

100%.

The diagnostic sensitivity and specificity have been obtained using 118 samples compared with the same method of a computer.

LIMITATIONS

- Reaction time is critical. If reaction time exceeds 2 minutes, drying of the reaction mixture may cause false positive result.
- Freezing the RF Latex Reagent will result in spontaneous agglutination.
- Intensity of agglutination is not necessarily indicative of relative RF concentration; therefore, screening reactions should not be graded.
- Increased levels of RF may be found in some diseases other than rheumatoid arthritis such as infectious mononucleosis, sarcodosis, lupus erythrematosus, Sjogren's syndrome.
- Certain patients with rheumatoid arthritis will not have the RF present in their serum.
- The incidence of false positive results is about 3-5 %.Individuals suffering from infectious mononucleosis, hepatitis, syphilis as well as elderly people may give positive results.
- Diagnosis should not be solely based on the results of latex method but also should be complemented with a Waaler Rose test along with the clinical examination.

REFERENCE VALUES

Up to 8 IU/mL. Each laboratory should establish its own reference range.

NOTES

1. Results obtained with a latex method do not compare with those obtained with Waaler Rose test. Differences in the results between methods do not reflect differences in the ability to detect rheumatoid factors.

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PPI008A01, Rev H (17.06.2017)

REF	Catalogue Number		Store at
IVD	For In-Vitro Diagnostic use		Caution
Σ	Number of tests in the pack		Read product insert before use
LOT	Lot (batch) number		Manufacturer
	Fragile, handle with care		Expiry date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		

Atlas Medical

CE

ANTISTREPTOLYSIN-O (ASO) LATEX SLIDE TEST

For the qualitative and quantitative measurement of antibodies to Antistreptolysin-O in human serum.

IVD *For in -vitro diagnostic and professional use only*

2°C 8°C Store at 2-8°C

INTENDED USE

ATLAS ANTISTREPTOLYSIN-O (ASO) latex slide Test is used for the qualitative and quantitative measurement of antibodies to Antistreptolysin-O in human serum.

INTRODUCTION

The group A ß-hemolytic streptococci produces various toxins that can act as antigens. One of these exotoxins streptolysin-O, was discovered by Todd in 1932.

A person infected with group A -hemolytic streptococci produces specific antibodies against these exotoxins, one of which is antistreptolysin-O. The quantity of this antibody in a patient's serum will establish the degree of infection due to the -hemolytic streptococcal.

The usual procedure for the determination of the antistreptolysin titer is based on the inhibitory effect that the patient's serum produces on the hemolytic power of a pre-titrated and reduced streptolysin-O. However, the antigen-antibody reaction occurs independently of the hemolytic activity of streptolysin-O. This property enables the establishment of a qualitative and quantitative test for the determination of the antistreptolysin-O by agglutination of latex particles on slide.

PRINCIPLE

ASO test method is based on an immunologic reaction between streptococcal exotoxins bound to biologically inert latex particles and streptococcal antibodies in the test sample. Visible agglutination occurs when increased antibody level, are present in the test specimen.

MATERIALS

MATERIALS PROVIDED

- ASO Latex Reagent: Latex particles coated with streptolysin O, pH, 8,2. Preservative
- ASO Positive Control(Red cap): Human serum with an ASO concentration > 200 IU/mL.Preservative
- ASO Negative Control (Blue cap) Animal serum. Preservative
- Reaction Slide.
- Stirring Sticks.

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer.
- Test Tubes 12x75mm.
- Test Tube Rack.
- Serological pipettes.
- High intensity light.
- Saline Solution, 0.9% NaCL.

PRECAUTIONS

- All reagents contain 0.1% (w/v) sodium azide as a preservative. Store all reagents at 2-8°C. **DO NOT FREEZE.**
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide build-up.
- For In Vitro diagnostic use.
- Positive and negative controls prepared using human serum found negative for hepatitis B surface antigen (HBsAg) and HIV-III by FDA required test; however, handle controls as if potentially infectious.

REAGENT STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2-8°C).
- **DO NOT FREEZE.**
- The ASO Latex Reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.

SPECIMEN COLLECTION AND STORAGE

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8(C and for 3 months at -20(C.
- For longer periods the sample must be frozen.
- As in all serological tests, hemolytic or contaminated serum must not be used.
- **DO NOT USE PLASMA.**

PROCEDURE

Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place 50 µL of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the ASO-latex reagent vigorously or on a vortex mixer before using and add one drop (50 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

Semi-quantitative method

1. Make serial two fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

QUALITY CONTROL

Positive and Negative Controls should be included in each test batch.

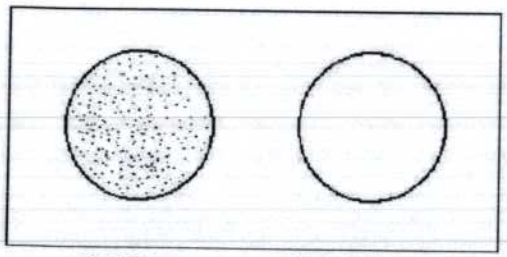
Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the ASO Negative Control and agglutination with large aggregates is observed with the ASO Positive Control.

RESULTS

A.QUALITATIVE TEST:

A negative reaction is indicated by a uniform milky suspension with no agglutination as observed with the ASO Negative Control.

A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared to the ASO Negative Control (Fig.1).



Positive Negative

Figure 1

B.QUANTITATIVE TEST

A positive reaction is indicated by any observable agglutination in the reaction mixture. Record the last dilution showing a positive reaction. Concentration of ASO can be determined by multiplying the last positive dilution factor of the sample with the concentration of the positive control (200 IU/ml).

The titer of the serum is the reciprocal of the highest dilution which exhibits a positive reaction.

IU/ml of sample = conc. of positive control (200) x specimen titer

DILUTION	IU/ml
1:1	200
1:2	400
1:4	800
1:8	1600
Etc.	

REFERENCE VALUES

Up to 200 IU/mL(adults) and 100 IU/mL (children < 5 years old)[6]. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity:

200 (±50) IU/ml.

PROZONE EFFECT

No prozone effect was detected up to 1500IU/ml.

SENSITIVITY

98%.

SPECIFICITY

97%.

INTERFERENCES

NON INTERFERING SUBSTANCES:

- Hemoglobin (10g/dl)
- Bilirubin(20mg/dl)
- Lipemia(10g/dl)

Other substances may interfere

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Fax: ++44 (0) 1223 858 524

PPI003A01
Rev H (09.09.2017)

REF	Catalogue Number		Store at
IVD	For In-Vitro Diagnostic use		Caution
Σ	Number of tests in the pack		Read product insert before use
LOT	Lot (batch) number		Manufacturer
	Fragile, handle with care		Expiry date
	Manufacturer fax number		Do not use if package is **damaged**
	Manufacturer telephone		

Техпластин-тест

ИНСТРУКЦИЯ
по применению набора реагентов для определения протромбинового времени на автоматических коагулометрах (с *жидким реагентом на 1000-2000 опр.*)

НАЗНАЧЕНИЕ

Техпластин-тест предназначен для оценки протромбинового времени свертывания на автоматических коагулометрах. Набор также возможно использовать и на полуавтоматических коагулометрах. Определение протромбинового времени используется для тестирования факторов протромбинового комплекса (II - протромбина, V, VII, X) и контроля лечения антикоагулянтами непрямого действия.

ХАРАКТЕРИСТИКА НАБОРА

Принцип метода. Тромбопластин (фактор III, тромбокиназа) превращает протромбин плазмы крови в присутствии ионов кальция в активный фермент тромбин, трансформирующий фибриноген плазмы крови в нерастворимый фибрин. Измеряется протромбиновое время - время образования фибрина в плазме крови в присутствии ионов кальция и тромбопластина (экстракта из мозга кролика).

Состав набора:

Техпластин (тромбопластин-кальциевая смесь из кроличьего мозга), суспензия 10 мл – 10 фл.

Международный индекс чувствительности (МИЧ) указан в Паспорте к набору.

Контрольная плазма в состав набора данной комплектации не входит. Для получения контрольных значений протромбинового времени свёртывания следует использовать пул бедной тромбоцитами плазмы, полученной от 3-5 практически здоровых людей.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ НАБОРА

Коэффициент вариации результатов определения протромбинового времени не превышает 10 %.

Допустимый разброс результатов определения протромбинового времени в одной пробе плазмы крови разными наборами одной серии не превышает 10 %.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а (ГОСТ Р 51609-2000).

Все реагенты, входящие в набор, используются только для применения *in vitro*.

Все компоненты набора в используемых концентрациях не токсичны. Компоненты набора проверены на содержание вирусов гепатита и ВИЧ.

При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы плазмы крови человека следует рассматривать как потенциально инфицированные, способные длительное время сохранять и передавать ВИЧ, вирус гепатита В или любой другой возбудитель вирусной инфекции.

Все использованные материалы дезинфицировать в соответствии с требованиями МУ-287-113.

ОБОРУДОВАНИЕ, МАТЕРИАЛЫ, РЕАГЕНТЫ

- Автоматический или полуавтоматический коагулометр;
- центрифуга лабораторная;
- физиологический (0,9 %) раствор натрия хлорида;
- перчатки резиновые хирургические.

Каталожный номер набора: 735

ООО фирма "Технология-Стандарт"
634037, Бийск, а/я 1351, тел./факс (3854) 22-99-37, 22-99-36, 22-99-39, 27-13-00

ПРИГОТОВЛЕНИЕ АНАЛИЗИРУЕМЫХ ОБРАЗЦОВ

Кровь для исследования забирают из локтевой вены в пластиковую или силиконированную пробирку, содержащую 3,8 % раствор натрия лимоннокислого трёхзамещенного (цитрата натрия), соотношение объемов крови и цитрата натрия - 9:1. Кровь центрифугируют при 3000-4000 об/мин (1200 g) в течение 15 мин. В результате получают бедную тромбоцитами плазму, которую переносят в другую пробирку, где хранят до проведения исследования.

Центрифугирование должно проводиться непосредственно после взятия крови, а отбор плазмы на исследование - сразу же после центрифугирования. Не допускается анализ плазмы, имеющей сгустки, гемолиз, избыток цитрата натрия и полученной более 2 ч назад, а также замороженной плазмы крови.

ПРИГОТОВЛЕНИЕ РЕАГЕНТОВ И ПРОВЕДЕНИЕ АНАЛИЗА

1. ПОДГОТОВКА РЕАГЕНТОВ К РАБОТЕ

Техпластин входит в комплект набора готовым к применению и не требует каких-либо разведений.

Получение контрольной плазмы:

Вариант 1: Бедная тромбоцитами плазма, полученная по описанному методу (*см. выше раздел «Приготовление анализируемых образцов»*) от 3-5 практически здоровых доноров, смешивается в равной пропорции.

Вариант 2: Может быть также использована коммерческая контрольная нормальная плазма, аттестованная по данному показателю.

Контрольную плазму следует использовать для получения нормативных данных и контроля активности Техпластина.

2. ПРОВЕДЕНИЕ АНАЛИЗА

Информацию об особенностях проведения анализа для определённой модели автоматического коагулометра можно получить в протоколе адаптаций, который опубликован на сайте www.tehnologia-standart.ru (в разделе «Протоколы адаптации»). Число определений может отличаться для разных конструкций автоматических коагулометров.

Определение контрольных (нормальных) показателей на полуавтоматических коагулометрах

1. В кювету полуавтоматического коагулометра внести 0,05 мл контрольной плазмы.
2. Инкубировать при температуре +37 °С в течение 1 мин.
3. Добавить 0,1 мл Техпластина, имеющего температуру +37 °С, и начать отсчет времени свертывания до образования фибрина.

Аналогично определить протромбиновое время на полуавтоматическом коагулометре в образцах плазмы больных.

Чтение результатов. Результат выражают по одному из следующих вариантов:

1. Протромбиновое время ***(ПВ)*** в секундах у больного с указанием значений, полученных при исследовании контрольной плазмы.

В норме протромбиновое время, измеренное на коагулометре, составляет **12-18 с**, при мануальной технике определения - **13-19 с**.

2. Протромбиновое отношение ***(ПО)***

В норме ***ПО*** составляет **0,9-1,3.**

3. Автоматический коагулометр способен вычислить протромбиновый показатель по Квику.

В норме **показатель по Квику** при использовании Техпластина **более 60 %**.

4. Международное нормализованное отношение (***МНО***) автоматический коагулометр вычисляет на основе значений ***ПО*** и международного индекса чувствительности (***МИЧ***), который указан в *Паспорте к набору*.

Нормальное МНО близко к **1,0**. При лечении антикоагулянтами непрямого действия обычно доводят МНО до 2,0-3,5, в зависимости от клинических показаний. Чем выше МНО, тем значительнее гипокоагуляция и тем чаще и опаснее геморрагические осложнения.

УСЛОВИЯ ХРАНЕНИЯ И ПРИМЕНЕНИЯ

Набор рассчитан на исследование **1000** образцов плазмы при использовании большинства автоматических и полуавтоматических коагулометров. При использовании некоторых коагулометров (при расходе жидкого Техпластина по 0,05 мл на 1 анализ) число определений увеличивается до **2000**.

Хранение набора должно проводиться при температуре +2... +8 °С в течение всего срока годности (**12 мес**). Допускается транспортировка при температуре до +25 °С в течение 30 сут, а также однократное замораживание.

После вскрытия флакона жидкий Техпластин можно использовать при температуре +37 °С не более суток, комнатной температуре (+18... +25 °С) - не более одной недели или не более 30 дней - при температуре +2... +8 °С.

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ТЕХНОЛОГИЯ СТАНДАРТ

Фибриноген-калибратор

ИНСТРУКЦИЯ
по применению набора калибраторов для определения концентрации фибриногена набором реагентов «МультиТех-Фибриноген»

НАЗНАЧЕНИЕ

Набор предназначен для получения калибровочных значений времени свёртывания при определении концентрации фибриногена в плазме крови **модифицированным** методом Clauss без предварительного разведения исследуемой плазмы на **автоматических и полуавтоматических коагулометрах**. Фибриноген-калибратор не предназначен для калибровки других методов определения концентрации фибриногена, в том числе набора «Тех-Фибриноген-тест».

ХАРАКТЕРИСТИКА НАБОРА

Принцип метода

Заключается в определении времени свертывания цитратной плазмы избытком тромбина (модифицированный метод Clauss). Время свертывания при этом пропорционально концентрации фибриногена, которую определяют по калибровочному графику.

Состав набора:

1. *Калибратор №1* (лиофильно высушенный) - 1 фл.
2. *Калибратор №2* (лиофильно высушенный) - 1 фл.
3. *Калибратор №3* (лиофильно высушенный) - 1 фл.
4. *Калибратор №4* (лиофильно высушенный) - 1 фл.
5. *Калибратор №5* (лиофильно высушенный) - 1 фл.

Концентрация фибриногена для каждого калибратора указана *в Паспорте к набору*.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ НАБОРА

Линейность определения: 0,9-10,0 г/л.

Коэффициент вариации результатов определения концентрации фибриногена при использовании набора калибраторов не превышает 10 %.

Допустимый разброс результатов определения концентрации фибриногена в одной пробе плазмы наборами калибраторов одной серии не превышает 10 %.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а (ГОСТ Р 51609-2000).

Все реагенты, входящие в набор, используются только для применения *in vitro*.

Все компоненты набора в используемых концентрациях не токсичны.

При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы плазмы крови человека следует рассматривать как потенциально инфицированные, способные длительное время сохранять и передавать ВИЧ, вирус гепатита В или любой другой возбудитель вирусной инфекции.

Все использованные материалы дезинфицировать в соответствии с требованиями МУ-287-113.

ОБОРУДОВАНИЕ, МАТЕРИАЛЫ, РЕАГЕНТЫ

- Коагулометр;
- дозаторы пипеточные на 1,0 мл;
- вода дистиллированная;
- перчатки резиновые хирургические;
- набор реагентов «МультиТех-Фибриноген» (заказывается дополнительно, кат. № 712 и кат. № 711).

Каталожный номер реагента: 714

ООО фирма "Технология-Стандарт"
656037, Барнаул, а/я 1351, тел./факс (3852) 22-99-37, 22-99-38, 22-99-39, 27-13-00

ПРИГОТОВЛЕНИЕ РЕАГЕНТОВ И ПРОВЕДЕНИЕ АНАЛИЗА

1. ПОДГОТОВКА РЕАГЕНТОВ К РАБОТЕ

В каждый из пяти флаконов калибраторов фибриногена внести по **1,0 мл** дистиллированной воды и растворить содержимое при комнатной температуре (+18... +25° С) и слабом покачивании в течение 15 мин. В результате получают образцы с указанной в *Паспорте к набору калибраторов* концентрацией фибриногена.

2. ПРОВЕДЕНИЕ АНАЛИЗА

Используя инструкцию для набора реагентов необходимо определить время свертывания разведённых калибраторов №1, №2, №3, №4 и №5.

Для построения калибровочной кривой необходим набор для определения фибриногена «МультиТех-Фибриноген» (заказывается дополнительно).

В зависимости от типа коагулометра существуют два варианта набора реагентов «МультиТех-Фибриноген» для автоматических (кат. № 712) и полуавтоматических (кат. № 711) коагулометров.

3. ЧТЕНИЕ РЕЗУЛЬТАТОВ

Время свертывания калибровочного образца плазмы составляет **5-100 с**, в зависимости от концентрации фибриногена.

УСЛОВИЯ ХРАНЕНИЯ И ПРИМЕНЕНИЯ

Набор рассчитан на выполнение не менее **10 калибровочных кривых** при расходе по **0,1** мл на одно исследование. Однако в большинстве ситуаций рекомендуется дублирование результатов для построения калибровочной кривой.

Хранение набора должно проводиться при температуре +2... +8 °С в течение всего срока годности набора (**15 мес**). Допускается транспортировка при температуре до +25 °С в течение 30 сут.

После разведения растворы калибраторов пригодны для построения калибровочной кривой в течение 4 часов при комнатной температуре. Разведённые калибраторы не следует замораживать.

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МультиТех-Фибриноген

ИНСТРУКЦИЯ
по применению набора реагентов для определения концентрации фибриногена (*для полуавтоматических коагулометров*)

НАЗНАЧЕНИЕ

Набор предназначен для количественного определения содержания фибриногена в плазме крови на **полуавтоматических коагулометрах**, без предварительного разведения исследуемой плазмы.

ХАРАКТЕРИСТИКА НАБОРА

Принцип метода

Заключается в определении времени свертывания цитратной плазмы избытком тромбина (модифицированный метод Clauss). Время свертывания при этом пропорционально концентрации фибриногена, которую определяют по калибровочному графику.

Состав набора:

1. Тромбин (лиофильно высушенный реагент, 500 ед. NIH) - 2 фл.
2. Растворитель для тромбина, 10,5 мл - 2 фл.

АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ НАБОРА

Линейность определения от 0,9 до 10,0 г/л.

Коэффициент вариации результатов определения концентрации фибриногена не превышает 10 %.

Содержание гепарина в плазме до 1,0 Ед/мл не влияет на результаты определения.

Допустимый разброс результатов определения концентрации фибриногена в одной пробе плазмы разными наборами одной серии не превышает 10 %.

МЕРЫ ПРЕДОСТОРОЖНОСТИ

Потенциальный риск применения набора – класс 2а (ГОСТ Р 51609-2000).

Все реагенты, входящие в набор, используются только для применения *in vitro*.

Все компоненты набора в используемых концентрациях не токсичны.

При работе с набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы плазмы крови человека следует рассматривать как потенциально инфицированные, способные длительное время сохранять и передавать ВИЧ, вирус гепатита В или любой другой возбудитель вирусной инфекции.

Все использованные материалы дезинфицировать в соответствии с требованиями МУ-287-113.

ОБОРУДОВАНИЕ, МАТЕРИАЛЫ, РЕАГЕНТЫ

- Центрифуга лабораторная;
- полуавтоматический коагулометр;
- дозаторы пипеточные на 0,1-0,2, 10,0 мл;
- вода дистиллированная;
- перчатки резиновые хирургические;
- набор калибраторов «Фибриноген-калибратор» (кат. № 714, производитель ООО фирма «Технология-Стандарт», заказывается дополнительно).

ПРИГОТОВЛЕНИЕ АНАЛИЗИРУЕМЫХ ОБРАЗЦОВ

Кровь для исследования забирают из локтевой вены в пластиковую или силиконированную пробирку, содержащую 3,8 % раствор натрия лимоннокислого трёхзамещенного (цитрата натрия), соотношение объемов крови и цитрата натрия - 9:1. Кровь центрифугируют при 3000-4000 об/мин (1200 g) в течение 15 мин. В результате получают бедную тромбоцитами плазму, которую переносят в другую пробирку, где хранят до проведения исследования.

Центрифугирование должно проводиться непосредственно после взятия крови, а отбор плазмы на исследование - сразу же после центрифугирования. Не допускается анализ плазмы, имеющей сгустки, гемолиз, избыток цитрата натрия и полученной более 2 ч назад, а также замороженной плазмы крови.

ПРИГОТОВЛЕНИЕ РЕАГЕНТОВ И ПРОВЕДЕНИЕ АНАЛИЗА

1. ПОДГОТОВКА РЕАГЕНТОВ К РАБОТЕ

1.1. Разведение тромбина

В один флакон с тромбином внести **10,0 мл** растворителя для тромбина и растворить содержимое при комнатной температуре (+18... +25 °C) и перемешивании в течение 5 мин. В результате получают раствор тромбина. Тромбин во втором флаконе разводят по необходимости.

1.2. Разведение калибратора фибриногена

В каждый из пяти флаконов калибраторов фибриногена (заказывается дополнительно) внести по **1,0 мл** дистиллированной воды и растворить содержимое при комнатной температуре и слабом покачивании в течение 15 мин. В результате получают калибраторы с указанной в *Паспорте к набору калибраторов* концентрацией фибриногена.

2. ПОСТРОЕНИЕ КАЛИБРОВОЧНОЙ КРИВОЙ

Для построения калибровочной кривой необходим набор калибраторов фибриногена «Фибриноген-калибратор» (кат. № 714; заказывается дополнительно).

2.1. В кювету коагулометра внести **0,1 мл** плазмы-калибратора №1.

2.2. Инкубировать при температуре +37 °C в течение 1 мин.

2.3. В ту же кювету добавить **0,2 мл** рабочего раствора тромбина, имеющего комнатную температуру и начать отсчет времени свертывания.

2.4. Аналогично определить время свертывания с плазмой-калибратором № 2, № 3, № 4 и № 5.

2.5. По полученным данным необходимо построить калибровочную кривую.

3. ПРОВЕДЕНИЕ АНАЛИЗА

3.1. В кювету коагулометра внести **0,1 мл** (*см. раздел "Приготовление анализируемых образцов"*) исследуемой плазмы и прогреть её в течение 1 мин при температуре +37 °C.

3.2. В ту же кювету добавить **0,2 мл** рабочего раствора тромбина, имеющего комнатную температуру и начать отсчет времени свертывания.

3.3. Используя результаты определения времени свёртывания, по калибровочной кривой определяют концентрацию фибриногена в исследуемом образце плазмы.

4. ЧТЕНИЕ РЕЗУЛЬТАТОВ

Время свертывания исследуемого образца плазмы составляет **4-100 с**, в зависимости от концентрации фибриногена и типа коагулометра. Диапазон определения концентрации фибриногена без дополнительного разведения составляет **0,9-10,0** г/л. Если результаты определения близки к 0,9 г/л или меньше (отсутствие регистрации сгустка), концентрацию фибриногена следует определить классическим методом Clauss набором реагентов «Тех-Фибриноген-тест» (кат. № 094 или кат. № 324) или аналогичным с разведением плазмы 1:5.

Концентрация фибриногена у здоровых людей находится в диапазоне от 2,0 до 4,0 г/л.

УСЛОВИЯ ХРАНЕНИЯ И ПРИМЕНЕНИЯ

Набор рассчитан на выполнение **100 анализов** при расходе раствора тромбина по **0,2** мл на одно исследование, или **200 анализов** при расходе раствора тромбина по **0,1** мл.

Допускается транспортировка при температуре до +25 °C в течение 30 сут.

Хранение набора должно проводиться при температуре +2... +8 °C в течение всего срока годности набора (**15 мес**).

Закрытый пробкой раствор тромбина можно хранить до 2-х недель при комнатной температуре и не более месяца при +2...+8 °C. Раствор тромбина при необходимости можно замораживать на срок до 30 суток при температуре (-16... -20 °C).

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