



Please consult the Product Package Insert before running the test

Sample Collection, Preparation, Storage and Volume

Q. What type of blood sample should be used with the PIFA[®] Heparin/PF4 Rapid Assay?

A. *FRESH Serum must be used with the PIFA[®] Heparin/PF4 Rapid Assay. **Do not use frozen or thawed specimens.***

Q. What type of collection tube should be used to collect the blood sample?

A. *Blood specimens **MUST** be collected in **Red Top Tubes ONLY**; Plain, Glass Red Top tubes OR Plastic, Red Top Tubes, with Clot Activator are required for collection. No other collection tubes should be used, including gel serum separation tubes (SST).*

NOTE:

- *Plasma should not be used with the PIFA[®] assay. Anticoagulated samples are not suitable for testing with this assay and **must not be used.***
- *Frozen, thawed, hemolyzed, icteric, lipemic (of an excessive nature), bacterial contaminated specimens, or controls from other test kits should not be used, and can produce erroneous results.*
- *Please also consult the “Limitations of the Procedure” section of the package insert for additional information.*

Q. Should a freshly drawn blood sample, collected in a Red Top tube, clot before centrifugation?

- A. *Yes, patient specimens must be allowed to clot before centrifugation.*
- **Plastic, Red Top Tubes with Clot Activator:** *Consult Collection Tube Manufacturer’s instructions for mixing protocols and recommended clotting times (clotting times range from thirty (30) to sixty (60) minutes).*
 - **Plain, Glass Red Top Tubes:** *Allow specimen to clot for approximately 30 minutes.*

NOTE: Centrifugation: *The specimen should be centrifuged at the end of the clotting period in strict accordance with the Collection Tube Manufacturer’s instructions for speed and duration of centrifugation.*



Q. At what time interval should the FRESH Serum specimen be processed off the clot after the draw?

- A. *In accordance with CLSI Guideline H18-A3, "Procedures for the Handling and Processing of Blood Specimens", serum should be separated from contact with cells within a **maximum of 2 hours** of the draw.*⁽¹⁾

Be sure to check the test tube manufacturer's instructions as the collection tube you are using may require that separation occur in less than 1 hour. For example, Greiner Vacuette Red Top serum tubes with clot activator require that "Serum Tubes should be centrifuged 30 minutes after blood collection to minimize post clotting (build up fibrin) in serum".⁽²⁾

IMPORTANT: Patient specimen should not stand on the clot for longer than 60 minutes after centrifugation or the PIFA[®] test result may be invalid.

Properly prepared serum that has been removed from the clot, but cannot be tested immediately, should be stored refrigerated (2-8°C; 36-46°F) for no longer than 72 hours.

Q. How should I store patient serum that cannot be tested immediately?

- A. *Serum that cannot be tested immediately should be stored at 2-8°C (36-46°F) for no longer than 72 hours, and must not be stored on the clot.*

Note: *Any refrigerated serum should reach an ambient temperature prior to performing the test.*

Serum stored at 2-8°C (36-46°F) for less than 72 hours should always be checked visually for bacterial growth and/or a cloudy appearance. Samples with bacterial growth will produce erroneous results; the bacteria may clog the pores in the membrane filter system in the test device, and could cause a negative sample to produce a positive test result.

Do not freeze serum samples; do not use serum that has been frozen. Freezing samples may cause at least two problems.

- 1. If not processed meticulously, freezing and thawing will decrease antibody activity, and could cause a positive sample to produce a negative test result.*
- 2. Freezing/thawing may also cause certain proteins to precipitate out of solution, and cause other microparticulates or debris to form. These particulates can clog the pores in the membrane filter system in the test device, and could cause a negative sample to produce a positive test result.*



Q: If too much or not enough specimen sample was added to the device, is the result still reliable?

A: No, an incorrect amount of specimen sample can affect the test result. Caution must be exercised so that the appropriate sample volume of fresh serum is used in the assay. Please consult the product insert for volume specifications.

Q: It appears that the sample volume required for the PIFA[®] Heparin/PF4 Rapid Assay has increased for product released in or after June 2008. Why is this?

A: Yes. ABI has modified the reagent formulation and membrane system to increase the vibrancy of the color produced by the test. Not only has this modification enhanced the clarity of results, but it has also decreased the time to assay completion. Please be sure to check Step 2 of the TEST PROCEDURE section of the product insert packaged with your box of PIFA[®] Heparin/PF4 Rapid Assays to verify the required volume of serum for your lot of product. Product released in or after June 2008 requires a 30 μ L serum specimen, whereas older product requires 20 μ L.

In an effort to call attention to the sample volume change, all PIFA[®] Heparin/PF4 Rapid Assay product that requires the 30 μ L sample volume has been packaged with a yellow sticker on the front panel that alerts the user to "Please review package insert – Sample Size Increase".

Q. Can plasma be used with the PIFA[®] test?

A: No, the PIFA[®] Heparin/PF4 Rapid Assay is designed for use with Serum only.

Plasma is obtained when blood is collected in a tube containing various anticoagulants. The chemical make-up of anti-coagulants will interfere with the reaction between the reagent and the sample. Additionally, plasma contains fibrinogen which may initiate non-specific microparticle aggregation.

Quality Control

Q: How does ABI recommend our laboratory initially validate the test?

A: It is the responsibility of the Laboratory to define internal validation protocols, as applicable, in accordance with good clinical and scientific laboratory practice. In lieu of established lab procedure, ABI recommends that 20-30 test evaluations be performed using well-characterized, fresh serum samples.



ABI offers 510(K)-cleared, serum panels to help facilitate the validation process. The Heparin/Platelet Factor 4 Antibody Serum Panels are assayed controls, intended for use as a serum control to monitor and evaluate precision and accuracy of the (qualitative) PIFA[®] Heparin PF4 Antibody Assay. The panel contains confirmed positive and negative members.

Contact us at 1.800.451.TEST or +1.856.848.2116 for additional information regarding the panels.

Q. What ongoing Quality Control measures should be taken once the PIFA assay has been adopted by our laboratory?

A. The PIFA[®] Heparin/PF4 Rapid Assay is a single-use system. ABI recommends that the laboratory analyzes a sample representative of the lot for Quality Control. It is also recommended that a positive and negative control be utilized in the following circumstances:

- 1) during the initial use of a new lot of PIFA[®] Heparin/PF4 Rapid Assay devices,*
and
- 2) after running 100 tests of the same lot of devices.*

Controls should be assayed as required by your laboratory's standard quality control procedures using the same procedure as the specimens. Use only confirmed Heparin/PF4 antibody positive and negative samples as controls. As mentioned earlier, ABI manufactures FDA-cleared Heparin/PF4 Antibody Serum Panels for use with the PIFA[®] Heparin/PF4 Rapid Assay. For information on product availability, contact 1-800-451-TEST or +1.856.848.2116. See package insert for additional information.

Q. How often do I have to run a confirmatory test since the PIFA[®] H/PF4 Rapid Assay is a screening test?

A. It is the responsibility of the Laboratory to define protocols, as applicable, in accordance with good clinical and scientific laboratory practice. The laboratory may choose to run a confirmatory test when a positive PIFA[®] test result is obtained or when a PIFA[®] test result is inconsistent with other clinical findings. Given that the PIFA[®] test is an immunoassay, a laboratory may decide to run a functional assay for confirmation.

The PIFA[®] Heparin/PF4 Rapid Assay is a screening test and should be used for the qualitative detection of antibodies directed against the Heparin/PF4 complex. Test results should, therefore, not be relied upon solely to identify antibodies to the Heparin/PF4 complex. Results obtained from the PIFA[®] Heparin/PF4 Rapid Assay should be interpreted along with clinical findings or other serological tests.



Q. How does one know that the test has been performed properly?

A. *The Laboratory Technician must carefully follow the Test Procedure noted within the Product Package Insert provided with each sleeve of tests.*

- *Inadequate incubation time, incomplete mixing, or improperly performed test procedures can produce erroneous results.*

The PIFA[®] Heparin/PF4 Rapid Assay provides an internal device control with each test run. The appearance of RED in the CONTROL Window indicates that the device has functioned as designed. If RED does not develop in the CONTROL Window within a maximum of 20 minutes after performing the test procedure, the test result is considered invalid.

Q. Is the 1 minute incubation time, noted in Step 3 of the PIFA[®] Test Procedure, critical to the proper functioning of the assay?

A. *Yes, care should be taken to complete all Steps of the Test Procedure according to the Package Insert and/or Pictorial Guide. Since the PIFA[®] method is based on a rate reaction which occurs between serum and reagent mixture in Step 3, the Laboratory Technician must use a timing device to help ensure that exactly one minute has elapsed before pulling up the Tower in Step 4.*

Q. Should the PIFA[®] Heparin/PF4 Rapid Assay be included in Proficiency Testing?

A. *It is the responsibility of the Laboratory to define Proficiency Testing procedures, as applicable, in accordance with good clinical and scientific laboratory practice. At present, there is limited to no availability of proficiency panels for heparin/PF4 antibody assays.*



Interpretation

Q. Does the intensity of the BLUE color vary in the TEST Result Window when a Negative result is obtained?

A. *Yes, color variation is sample dependent. The rate reaction and aggregation of microparticles differ among samples causing more or less blue to appear in the TEST Result window.*

Q. A Negative Result is read when ANY BLUE color appears in the TEST result window and RED appears in the Device CONTROL window. Will the BLUE color vary in intensity?

A. *The intensity of the BLUE color may vary from patient-to-patient. Any trace of BLUE in the TEST Window along with RED in the CONTROL Window is considered a NEGATIVE result. NO BLUE in the TEST Window indicates a POSITIVE Result.*

Q. What is the maximum time interval that I should wait for RED to appear in the Device CONTROL window?

A. *Since flow rate is sample dependent, the time interval for RED to develop in the CONTROL window ranges from approximately 1 minute to a maximum of 20 minutes; the latter time estimate often applies to high titer specimens. If RED fails to appear in the CONTROL window beyond the 20-minute mark, the test should be considered Invalid.*

Also, in June 2008, ABI released the second generation of the PIFA[®] Heparin/PF4 Rapid Assay that integrates a modified reagent formulation and membrane system. As a result, you will find that the time to result is significantly less than the previous generation. Negative samples are often completed in one minute and positives rarely take longer than 10 minutes to develop.

Q. Once RED appears in the Device CONTROL Window, how long is the Test result stable?

A. *One (1) hour.*



Device Storage

Q: How should the test kits be stored?

A: *Store the tests as packaged in their sealed pouch at 2 - 8°C (36-46°F) until use. Do not freeze the tests.*

Q: Is it important to wait at least 30 minutes after removing the device from refrigeration before running the assay?

A. *YES – When a test device is removed from refrigeration, keep it within the sealed pouch and allow it to equilibrate to Room Temperature (15 to 30°C (59-86°F)) for at least 30 minutes prior to use. Remove the kit from the refrigerated unit and take the required amount of test devices out of the kit packaging. Keep the test device within the sealed pouch.*

If the ambient temperature in the laboratory is closer to the lower end of the Room Temperature range, be sure that the device is not cold to the touch when you are initiating the assay procedure. The colder the reagent/sample mixture, the more slowly the antibody/antigen reaction occurs.

Q: If our laboratory countertop tends to be colder than the ambient temperature, could it impact the functioning of the device?

A. *Yes, If your countertop is made from material that stays colder than the ambient temperature, place a flat material between the device and the bench top. A mouse pad may be a good option. Ensure that the device will remain level and you will be able to easily slide the device from side-to-side, as noted in the test procedure within the package insert.*

Q: Once a PIFA[®] Heparin/PF4 Rapid Assay device is removed from refrigeration, can it be returned to refrigeration if it is not used?

A: *Yes, if the device remains in the sealed pouch while equilibrating to room temperature, it can be returned to refrigeration within a maximum of 8 hours.*



Q: If the pouch has been opened, should the test be used immediately?

A: YES – As long as the device has been able to warm to Room Temperature in the pouch, the device should be used immediately once it is opened. Humidity in the air can affect the integrity of the test. If the test has not been able to warm to Room Temperature and is removed from the pouch, the test should not be used.

If a pouch is found to be punctured when first removed from PIFA[®] packaging, do not use the test inside and contact Customer Service at 1.800.451.TEST (8378).

Q: Can the device be used beyond the expiration date on the bottom of the device?

A: No. The device should not be used past its expiration date under any circumstances.

Q: What is the shelf life?

A: When stored as indicated in the package insert, the shelf life is 12 months from the date of manufacture. For your convenience, this expiration date is labeled on the bottom of each individual device, as well as on the outer packaging for the sleeve of 6 devices.



REFERENCES:

- 1 CLSI (Clinical and Laboratory Standards Institute) H18-A3: Procedures for the Handling and Processing of Blood Specimens; Approved Guideline -- 3rd ed. (2004) (ISBN 1-56238-555-0).
- 2 Greiner Vacuette is a registered trademark of Greiner Vacuette North America, Inc. Monroe, NC.