

**Sample Collection, Preparation & Storage****Q. What type of tube should I collect the blood sample in?**

- A. The PIFA<sup>®</sup> Heparin/PF4 Rapid Assay is designed for use with **Serum only**. As a result, **only Red Top serum collection tubes** should be used to obtain patient specimens. Plain, glass Red Top tubes OR plastic, Red Top Tubes, with or without Clot Activator, may be utilized with the PIFA<sup>®</sup> test; SST Tubes are not recommended.

*Caution must be exercised so that the appropriate 20µL sample volume of fresh serum is used in the assay.*

**NOTE:** Plasma should not be used with the PIFA<sup>®</sup> assay. Anticoagulated samples are not suitable for testing with this assay and must not be used. Hemolyzed, icteric, lipemic (of an excessive nature), bacterial-contaminated specimens, controls from other test kits, or samples from patients with multiple myeloma, should not be used, and can produce erroneous results. It is not recommended that frozen patient samples be used with the test; extreme care must be taken when preparing, freezing and thawing a serum specimen in order to mitigate the formation of particulates and prevent protein break down and other factors that will influence the validity of the test result.

**Q. Should a freshly drawn blood sample, collected in a Red Top serum collection tube, sit before spinning down?**

- A. Yes. When using plastic, Red Top tubes with clot activator, allow the sample to sit approximately 15 minutes to facilitate clotting. If plain, Red Top glass tubes are used, the sample should sit for approximately 30 minutes. After the specimens have clotted, spin down for 10 minutes at 1500 g.

**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.

*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 as they 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 done 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 20 $\mu$ L sample volume of fresh serum is used in the assay.*

**Q. Can plasma be used with the PIFA<sup>®</sup> test?**

*A: No, the PIFA<sup>®</sup> 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 microparticle aggregation non-specifically.*

**Quality Control**

**Q: How does Akers 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, Akers would recommend that 20-30 test evaluations be performed using well-characterized, fresh serum samples.*

*Well-characterized frozen samples, for the purposes of validation ONLY, can be used with the test as long as the sample preparation and freezing processes mitigate the formation of particulates and protein break down. Samples should be flash frozen and stored at  $-70^{\circ}\text{C}$  ( $-94^{\circ}\text{F}$ ), and should only be subjected to one freeze-thaw cycle. A quick-thaw procedure should be used (thaw in a water bath with temperatures between  $30-37^{\circ}\text{C}$  ( $86-98^{\circ}\text{F}$ )).*

*Akers offers a serum panel, designed for Research Use Only, to help facilitate the validation process. Contact Akers' Technical Assistance at 1.800.451.TEST for additional information on the panel.*

**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 confirmatory tests when a positive PIFA<sup>®</sup> test result is obtained, or when a PIFA<sup>®</sup> test result is inconsistent with other clinical findings.*

*The PIFA<sup>®</sup> Heparin/PF4 Rapid Assay should be used for the qualitative detection of antibodies directed against the Heparin/PF4 complex, and should be used as a screening test. Test results should, therefore, not be relied upon solely to identify antibodies to the Heparin/PF4 complex. Positive or negative test results obtained from the PIFA<sup>®</sup> 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 follow all 5 Steps in the Test Procedure noted within the Product Package Insert and/or Pictorial Guide provided with each sleeve of tests.*

*The PIFA<sup>®</sup> Heparin/PF4 Rapid Assay also includes an Internal/Procedural Control. When RED appears in the CONTROL Window, the device has functioned properly.*

**Q. Is the 1 minute incubation time, noted in Step 3 of the PIFA<sup>®</sup> Test Procedure, critical to the proper functioning of the assay?**

*A. Care should be taken to complete all 5 Steps of the Test Procedure according to the Package Insert and/or Pictorial Guide. However, since the PIFA<sup>®</sup> 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 4a.*

**Q. Should the PIFA<sup>®</sup> 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.*

**Readability**

**Q. Why does the intensity of the BLUE color vary in the TEST Result Window?**

*A. Coloration 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. Does it matter that the Blue color is very faint?**

*A. No. The intensity of the BLUE color in the test window will vary. Any trace of BLUE in the TEST window (ranging from a darker, bright Blue to a very faint tint of Blue) along with a RED in the CONTROL window is considered a Negative 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 4 minutes to a maximum of 50 minutes; the latter time estimate often applies to strong positive specimens.*

*If RED fails to appear in the CONTROL window beyond the 50-minute mark, the test should be considered Invalid.*

**Q. How long is the Test result stable for once RED appears in the Device CONTROL Window?**

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. When a test device is removed from refrigeration, keep device within 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.*

**Q: If the pouch has been opened, should the test be used right away?**

A: *Because humidity in the air can affect the integrity of the test, it is recommended that the test be used immediately once the pouch has been opened. Likewise, if a pouch is found to be punctured, do not use the test inside.*

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

A: *If the device remains in the sealed pouch while equilibrating to room temperature, it can be returned to refrigeration within a maximum of 4-hours.*

**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.*



**Akers Biosciences**

PIFA<sup>®</sup> Heparin / PF4 Rapid Assay

Frequently Asked Questions

**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.*