PENTAPHARM LTD

Pefakit® PiCT® DxS Diluent

REF **505-02**

Research Use Only

Intended Use and Application

Pefakit® PiCT® DxS Diluent is intended to be used with Pefakit® PiCT® (Prothrombinase induced Clotting Time) for determination of total heparin. Results should not be used for diagnostic purposes.

Introduction

Platelet factor 4 (PF4) is a physiological heparin antagonist, synthesized in megakaryocytes and stored in the α-granules of platelets. Heparin treatment increases the level of PF4 in plasma 10-30 folds, due to a release of PF4 bound to the endothelial cell surface [1]. Dextran sulfate treatment of plasma frees heparin bound to PF4.

Principle of the Method

PiCT® Activator (R1) is reconstituted with Pefakit® PiCT® DxS Diluent. The plasma sample is mixed with the reagent containing a combination of a defined amount of activated factor X (FXa), phospholipids, RVV-V, an enzyme from the venom of the snake *Daboia russelli* specifically activating factor V [2] and DxS. During the incubation period of 180 s the DxS frees the heparin from the PF4-heparin complex resulting in an additional FXa inhibition depending on the amount of AT-heparin complexes and/or direct FXa and/or FIIa inhibitors present in the sample. Following recalcification the prothrombinase complex is formed using the residual FXa, phospholipids, factor Va generated from factor V present in the sample and free calcium ions. The time until the detection of clotting is recorded.

PiCT® without DxS measures the actual anticoagulation potential of free heparin in a plasma sample. In contrast PiCT® with DxS measures the total anticoagulation potential of heparin, including the potential of the PF4-bound and in vivo not anticoagulative active heparin. Therefore PiCT® measurement without use of DxS might be interpreted as lower concentrations of heparin compared with the ones obtained by PiCT® measurements with DxS, depending on the amount of PF4-heparin complexes present in the patients plasma at the moment of blood sampling.

Reagent

Content
PiCT® DxS Diluent
(0.75 ‰ Dextran sulfate in deionized water)
3 vials containing 2.5 ml
Ready for use

Attention: allow reagent to adapt to room temperature for 30 min and mix well before use

Materials required but not provided

- Pefakit® PiCT® [REF 505-01]
- Pefakit® PiCT® Calibrators LMWH [REF 505-11]
  Pefakit® PiCT® Controls LMWH [REF 505-21]
  Pefakit® PiCT® Calibrators UFH [REF 505-12]
  Pefakit® PiCT® Controls UFH [REF 505-22]
- Calibrated pipettes (50 – 2000 µl)
- Automated or semi-automated coagulation instruments which employ mechanical or optical detection methods

  Note: When using automated or semi-automated coagulation analyzers refer always to manufacturer's operator manual or ask for a detailed adaptation protocol.

Storage and Stability

The test kit may be used up to the expiry date given on the label when stored unopened at 2 - 8°C.

Stability of the reagents after reconstitution:

Stability	
2 - 8°C	3 days
15°C	48 hours (on-board)
25°C	12 hours (on-board)

Procedure

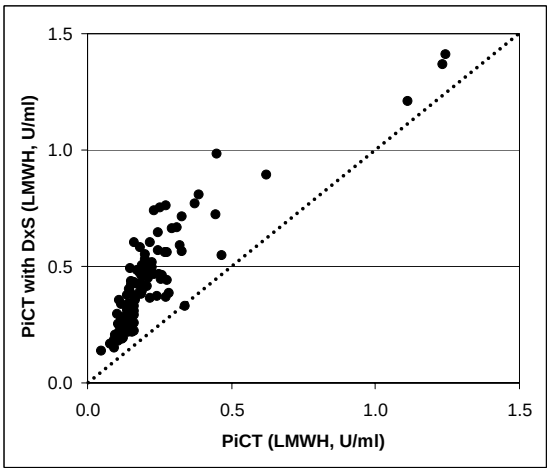
For determination of total heparin the PiCT® Activator (R1) is reconstituted with 2.0 ml Pefakit® PiCT® DxS Diluent.

The test procedure for Pefakit® PiCT® should be carried out according to the corresponding box insert instructions.

Consequently calibration and controls should been run also with the DxS containing PiCT®.

Expected Values

A collective of 125 patients under low molecular weight heparin (LMWH) therapy was tested. The LMWH concentrations using PiCT® without DxS are significantly lower compared with the concentrations found using PiCT® with diluent containing DxS ($p < 0.05$).



Bibliography

1. Mixon TA, Dehmer MD. Recombinant platelet factor 4 for heparin neutralisation. Sem Thromb Hemost 2004, 30: 369-77.
2. Calatzis A, Spannagl M, Gempeler-Messina P, Kolde HJ, Schramm W, Haas S. The prothrombinase induced clotting test: A new technique for the monitoring of anticoagulants. Haemostasis 2000; 30 (Suppl. 2): 172-174.

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