Introduction

Activated and aggregated platelets play a key role in the pathogenesis of cardiovascular disease. An important part of antiplatelet therapy in cardiovascular disease is aspirin (acetylsalicylic acid, ASA), which has been known for many years to have antiplatelet activity. Its effectiveness varies among individuals. It has been estimated that 10-20% of aspirin users have recurrent thrombotic events—a phenomenon commonly referred to as aspirin resistance. Activated platelets produce thromboxane A2 (TXA2), a potent vasoconstrictor and inducer of platelet aggregation. Therefore, measurement of stable metabolites of TXA2, such as urinary 11-dehydro thromboxane B2 (11dhTxB2), is a means of quantitating TXA2 production by platelets and thus a direct way to analyze the effectiveness of aspirin therapy.

This study presents the performance evaluation of an ultra-sensitive latex-enhanced immunoturbidimetric assay (ITA) to determine levels of 11dhTxB2 in human urine applied to the fully automated RX daytona plus analyzer. This is of value for the assessment of aspirin (ASA) effect in apparently healthy individuals post ingestion.

Methodology

**Analytical parameters**
- **Sensitivity**: The Limit of Blank was established by testing 120 replicates of a 0.9% saline solution. Generated in C.L.S.I. guideline EP17-A ‘Protocols for Determination of Limits of Detection and Limits of Quantitation’ were then applied to the data generated to determine the assay Limit of Blank.
- **Linearity**: Linearity was determined by assessment of replicates of a mixed pools dilution series of 11dhTxB2 spiked urine samples with an upper limit of 5000 pg/mL.
- **Sensitivity**: A blank and calibration standards were tested by staining one lot of reagent unaltered on the RX daytona plus analyzer for a period of 3 weeks. Using assays were carried out in which reagents were stored at +37°C for 2 weeks and then performance compared to fresh material.
- **Precision**: Within-run and total precision were assessed by testing samples at defined medical levels, 2 replicates twice a day for 20 days.

**Method comparison**: a method comparison study was conducted by testing 74 urine samples using the assay and another commercially available 11-dehydro thromboxane B2 assay.

Results

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**Method comparison**

- **Sensitivity and linearity**
- **Precision**
- **Sensitivity**
- **Linearity**

**Conclusion**

- **DEVELOPMENT OF AN ASSAY KIT FOR THE DETERMINATION OF 11-DEHYDRO THROMBOXANE B2 IN URINE REQUIRING LOW REAGENT AND SAMPLE VOLUMES ON THE RX daytona plus ANALYSER**

Activated and aggregated platelets play a key role in the pathogenesis of cardiovascular disease. An important part of antiplatelet therapy in cardiovascular disease is aspirin (acetylsalicylic acid, ASA), which has been known for many years to have antiplatelet activity. Its effectiveness varies among individuals. It has been estimated that 10-20% of aspirin users have recurrent thrombotic events—a phenomenon commonly referred to as aspirin resistance. Activated platelets produce thromboxane A2 (TXA2), a potent vasoconstrictor and inducer of platelet aggregation. Therefore, measurement of stable metabolites of TXA2, such as urinary 11-dehydro thromboxane B2 (11dhTxB2), is a means of quantitating TXA2 production by platelets and thus a direct way to analyze the effectiveness of aspirin therapy.

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  - **Linearity**: Linearity was determined by assessment of replicates of a mixed pools dilution series of 11dhTxB2 spiked urine samples with an upper limit of 5000 pg/mL.
  - **Sensitivity**: A blank and calibration standards were tested by staining one lot of reagent unaltered on the RX daytona plus analyzer for a period of 28 days. Stressing studies were carried out in which reagents were stored at +37°C for 2 weeks and then performance compared to fresh material.
  - **Precision**: Within-run and total precision were assessed by testing samples at defined medical levels, 2 replicates twice a day for 20 days.

**Results**

- **Sensitivity and linearity**
- **Precision**

**Conclusion**

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