Additional Material

1. Micropettes and a multichannel micropipette or multistep pipette, pipette tips
2. Glass or plastic test tubes for diluting the samples.
3. Laboratory bottles or beakers and graduated cylinders for diluting wash and dilution buffer
4. Distilled or deionised water
5. Absorbent paper towels
6. Microtitre plate washer (alternatively, washing can be performed manually using a multichannel pipette)
7. Microtitre reader equipped with a 450 nm filter and, if possible, a 620 nm reference filter
8. Incubator (37°C)

Samples

Use fresh EDTA or citrated plasma samples. Centrifuge the blood within 30 minutes after the puncture at 2000 g for 30 min. at 4°C (preferably in a cold centrifuge with swing out rotor). Immediately after centrifugation, plasma should be carefully pipetted off. Plasma aliquots should be stored at a temperature below -30°C. The total time between blood collection and plasma freezing should not exceed 90 min. Thawing and refreezing of plasma aliquots is not recommended. Thawing for assay is achieved rapidly using a waterbath at 37°C. After thawing, plasma samples are placed in a crushed ice-water mixture until analysis.

Test Performance

Preparations and Stability of Reagents

All reagents must be at room temperature before use.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume/bottle</th>
<th>Additions</th>
<th>Bench Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution buffer</td>
<td>20 mL</td>
<td>30 mL distilled H₂O</td>
<td>4-8 weeks at 2-8°C</td>
</tr>
<tr>
<td>Wash buffer</td>
<td>20 mL</td>
<td>230 mL distilled H₂O</td>
<td>4-8 weeks at 2-8°C</td>
</tr>
<tr>
<td>POX AB (concentrate)</td>
<td>0.3 mL</td>
<td>Dilute 1:50 with POX dilution buffer: for 8 test wells: mix 20 µL conjugate with 1000 µL POX dilution buffer</td>
<td>working dilution: RT: 4 hours</td>
</tr>
<tr>
<td>Standard and Controls</td>
<td>-</td>
<td>1 mL distilled H₂O</td>
<td>Aliquot at -70°C: 8 weeks RT: 4 hours</td>
</tr>
<tr>
<td>Urokinase</td>
<td>-</td>
<td>12 mL dilution buffer</td>
<td>Aliquot at -70°C: 8 weeks RT: 4 hours</td>
</tr>
</tbody>
</table>

RT = Room Temperature
POX AB = Peroxidase conjugated Antibody

Microtitre Plate

Reseal unused strips in aluminium foil bag. Store at 4°C for up to 8 weeks.

Sample Dilutions

Dilute control 1: 5
Dilute samples 1: 2
60 µL control + 240 µL dilution buffer
150 µL sample + 150 µL dilution buffer
STANDARD DILUTIONS

Prepare serial dilutions (1:2 to 1:8) of the standard in dilution buffer according to the following protocol. C = concentration according to label

<table>
<thead>
<tr>
<th>Tube</th>
<th>Dilution factor</th>
<th>Dilution buffer</th>
<th>Conc. % of normal plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NT</td>
<td>0.25 mL Standard</td>
<td>C</td>
</tr>
<tr>
<td>B</td>
<td>1:2</td>
<td>0.25 mL Standard</td>
<td>0.25 mL C/2</td>
</tr>
<tr>
<td>C</td>
<td>1:4</td>
<td>0.25 mL from B</td>
<td>0.25 mL C/4</td>
</tr>
<tr>
<td>D</td>
<td>1:8</td>
<td>0.25 mL from C</td>
<td>0.25 mL C/8</td>
</tr>
<tr>
<td>E</td>
<td>-</td>
<td>-</td>
<td>0.25 ml 0</td>
</tr>
</tbody>
</table>

ASSAY PROCEDURE

Over view of assay procedure

<table>
<thead>
<tr>
<th>Time table</th>
<th>Summary of procedure</th>
<th>time required</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation of plate: binding of urokinase</td>
<td>100 µL</td>
<td>1 hour</td>
<td>37°C</td>
</tr>
<tr>
<td>wash 5 times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent, Standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample handling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Sample - incubation</td>
<td>100 µL</td>
<td>2 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>wash 5 times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. POX AB – incubation</td>
<td>100 µL</td>
<td>2 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>wash 5 times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Substrate - incubation</td>
<td>100 µL</td>
<td>15 minutes</td>
<td>RT</td>
</tr>
<tr>
<td>Stop solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Read absorbances</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time total: min.</td>
<td></td>
<td></td>
<td>6½-7½ +</td>
</tr>
</tbody>
</table>

1. MICROTTRE PLATE PREPARATION
Pipette 100 µL of the reconstituted urokinase to each well. Incubate the plate for 1 hour at 37 °C.

2. WASH PLATE
Wash required strips by adding 200 µL of wash buffer to the wells and tip out the contents. Wash the strips four times further with wash buffer. Tap strips on absorbant paper and make sure the wells are completely dry.

3. SAMPLE/STANDARD/CONTROLS ADDITION
Pipette 100 µL of the samples/standard/controls into separate wells. For zero standard, pipette buffer only into the well. Running standard/sample/controls in duplicate is recommended.

4. SAMPLE INCUBATION
Cover the plate with a fresh plastic foil and incubate for 2 hours at 37 °C.

5. WASH PLATE
Wash five times as described in step 2.

6. POX ANTIBODY ADDITION
Pipette 100 µL of POX antibody to all wells containing sample, controls or standards.

7. POX ANTIBODY INCUBATION
Cover the plate with a fresh plastic foil and incubate for 2 hours at 37 °C.

8. WASH PLATE
Wash five times as described in step 2.

9. SUBSTRATE
Pipette 100 µL of TMB substrate to all wells. Incubate for 15 minutes at room temperature. There should be a colour change from clear to blue.

10. STOP
Pipette 100µL of stop solution to all wells. The colour of the endproduct should be yellow.

11. READ
Measure absorbances at 450 nm (with 620 nm reference filter if available). Read absorbances within one hour after the addition of the stop solution.

NOTES

Be sure to prepare all reagents before proceeding with the assay. It is critical to keep the time necessary for pipetting standards and samples to a minimum and avoid delays.

Be sure to wash the plate thoroughly and completely remove any residual wash buffer after each wash cycle. Insufficient washing may lead to erroneously high values and incomplete removal of wash buffer to irregularities due to the dilution of added reagents.

As mentioned use a multistepper to add peroxidase conjugate, TMB substrate and stop solution.

TEST EVALUATION

CONSTRUCT A STANDARD CURVE
Construct a graph of the standard curve. An example of a typical curve is given below. A standard curve must be constructed with each assay.

DETERMINATION OF SAMPLE CONCENTRATION
Locate the absorbance for each sample on the curve and read the corresponding value from horizontal axis. Do not forget to multiply by the dilution factor (5) for the control (2) for the samples, prediluted before adding to the plate.

EVALUATION OF RESULTS

The results are measured as a percentage of normal pooled human plasma. The validity of the test may be checked on the basis of the calculated control values. Lowered PCI values have been detected in patients with disseminated intravascular coagulation and liver disease, whereas increased levels have been detected in survivors of MI.

TEST CHARACTERISTICS

This test system measures active PCI antigen. The inter- and intra-assay variations are less than 10 % and 5 %, respectively.

STABILITY AND STORAGE

All the components of the kit should be stored at 2…8°C and can be used until the indicated expiry date on the vial labels. For storage of samples see above.

SPECIAL PRECAUTIONS

Potentially hazardous material. Donor plasma used in this kit was tested by internationally approved methods for the presence of antibodies to HIV and hepatitis B virus and found to be negative. However, all human blood products should be handled as potentially infectious material.

The stop solution (H2SO4) can cause skin irritations, wash with plenty of water if spill on the skin.

LITERATURE


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