Thrombo-Wellcotest® is intended for the rapid semi-quantitative detection of fibrin/fibrinogen degradation products (FDPs). Thrombo-Wellcotest® has been categorized as a laboratory diagnostic kit under the Clinical Laboratory Improvement Act (CLIA). 2. SUMMARY AND EXPLANATION OF THE TEST Naturally occurring fibrin/fibrinogen degradation products were first demonstrated by Ferri and Ferreira 1 in certain pathological sera, subsequently the development of a haemagglutination inhibition immun assay 2,3 has made it possible to detect the presence of FDP at a sensitivity of 0.01 µg/ml. Thrombo-Wellcotest is particularly useful as a screening aid in the diagnosis of disseminated intravascular coagulation (DIC) and in the management of patients who have undergone renal dialysis treatment. Thrombo-Wellcotest is a simple and rapid test which allows the routine investigation of all patients at special risk. The test is designed to be performed after the patient has been adequately prepared and that in the presence of fibrin/fibrinogen degradation products (FDP) an increased level of fibrinogen deposition or thrombosis and coagulation have been demonstrated in pulmonary embolism.

The resting level of approximately 5 µg/ml in healthy persons, exceptionally high levels have been demonstrated in pulmonary disease, with a haemagglutination-inhibition immunoassay 13,14,15. Thrombo-Wellcotest is capable of detecting the presence of all the components of fibrin/fibrinogen degradation products (FDP). Thrombo-Wellcotest are extremely clear cut and can easily be recognised under any normal conditions of lighting. Granularity may be detected in negative patterns, depending on the visual acuity of the operator. Due to a difference in the liquid properties of serum and urine, the volume of one drop of serum is less than the volume of one drop of urine as dispensed by the pipettes provided with the sample kit. The amounts for preparing for the 1/5 dilutions of serum and urine are not identical. Semi-quantitative Procedure for Serum Samples 1. Prepare a 1/5 dilution of the serum sample by adding 0.2 ml of serum to 0.5 ml of saline buffer in a test tube. 2. Prepare a doubling dilution series from 1/10 to 1/120, or as specified. 3. Transfer 1 drop of each dilution starting from 1/320 to separate positions on the glass slide, using disposable pipette and built-up. 4. Mix the latex suspension thoroughly by shaking vigorously through three or four times and then let stand for 15 to 20 minutes before reading. 5. Mix the latex suspension thoroughly by shaking vigorously through three or four times then let stand for 15 to 20 minutes before reading. 6. Rotate the slide slowly for exactly two minutes while looking for obvious agglutination. 7. The end point is the last serum dilution which shows obvious agglutination.

LATEX SENSITISATION

3 ml of a 0.75% suspension of polylysine in 0.15 M NaCl is added to 1 liter of distilled water. The suspension is mixed with 15 ml of a 20% suspension of bovine serum albumin to 1 liter of distilled water. The mixture is dialyzed against distilled water for 24 hours to extract impurities. The concentration of bovine serum albumin is adjusted to 0.01%.

1. Add 1 ml of latex suspension (1/20 final concentration) to test tubes 1 and 2 respectively, similarly identify two of the rings on the slide for a positive reaction. 2. Using one of the disposable mixing rods stir each of the sample tubes and reagents thoroughly before testing. 3. Use one of the disposable mixing rods to mix the contents of two test tubes each. In serial dilution test tube 1 and 2 are identified respectively. 4. Mix the contents of two test tubes each. In serial dilution test tube 2 to position 2 of the reaction slide and one drop of test tube 1 to position 1 (pipette the liquids in that order). 5. Using the dropper provided with the bottle of saline buffer, place 0.75 ml of saline buffer in each test tube. 6. The reaction slide for two minutes. If the reaction is allowed to continue for longer false results may occur due to drying out of the mixture on the slide.

QUALITATIVE RESULTS

1. Ensure the sample is free from leukocytes or precipitates. Prepare a dilution of urine sample as follows:– Take small glass or plastic test tube, add one drop of the serum sample to test tube 2, place one drop of saline buffer to each of the test tubes 1 and 2 respectively, similarly identify two of the rings on the slide for a positive reaction. 2. Using one of the disposable mixing rods stir each of the sample tubes and reagents thoroughly before testing. 3. Use one of the disposable mixing rods to mix the contents of two test tubes each. In serial dilution test tube 1 and 2 are identified respectively. 4. Mix the latex suspension thoroughly by shaking vigorously three or four times and then add one drop of the diluted serum sample to test tube 1. 5. Mix the contents of two test tubes each. In serial dilution test tube 2 to position 2 of the reaction slide and one drop of test tube 1 to position 1 (pipette the liquids in that order). 6. Rotate the slide slowly for exactly two minutes while looking for obvious agglutination. 7. The end point is the last serum dilution which shows obvious agglutination.

The Thrombo-Wellcotest® Kit should be stored at 2 to 8°C when the reagents will return full reactivity at least until the date displayed on the original container. A slight change in the liquid properties of serum and urine, the volume of one drop of serum is less than the volume of one drop of urine as dispensed by the pipettes provided with the sample kit. The amounts for preparing for the 1/5 dilutions of serum and urine are not identical. Semi-quantitative Procedure for Urine Samples 1. Prepare a 1/320 dilution of the urine sample by adding 0.1 ml of serum to 0.9 ml of saline buffer in a test tube. 2. Prepare a doubling dilution series from 1/128 to 1/2048, or as specified. 3. Transfer 1 drop of each dilution starting from 1/320 to separate positions on the glass slide, using disposable pipette and built-up. 4. Mix the latex suspension thoroughly by shaking vigorously through three or four times then let stand for 15 to 20 minutes before reading. 5. Mix the latex suspension thoroughly by shaking vigorously through three or four times then let stand for 15 to 20 minutes before reading. 6. Rotate the slide slowly for exactly two minutes while looking for obvious agglutination. 7. The end point is the last serum dilution which shows obvious agglutination.

READINGS OF RESULTS

Note. All numerical values shown below should be considered to be quotable. A positive reaction is indicated by a slight to moderate visible clumping of the latex particles (Figure 2). The speed of appearance and quality of agglutination are important in establishing the correctness of the antigen-antibody combination. If reading the results in some plumbing systems to form explosive salts. The method is to autoclave for 15 minutes at 121°C; disposables should be autoclaved or incinerated. All other disposable items should be autoclaved or incinerated. The presence of leukocytes or precipitates in the serum sample may be detected by examining the serum sample under a microscope. Due to a difference in the liquid properties of serum and urine, the volume of one drop of serum is less than the volume of one drop of urine as dispensed by the pipettes provided with the sample kit. The amounts for preparing for the 1/5 dilutions of serum and urine are not identical. Semi-quantitative Procedure for Serum Samples 1. Prepare a 1/20 dilution of the serum sample by adding 0.1 ml of serum to 9.9 ml of saline buffer in a test tube. 2. Prepare a doubling dilution series from 1/10 to 1/120, or as specified. 3. Transfer 1 drop of each dilution starting from 1/320 to separate positions on the glass slide, using disposable pipette and built-up. 4. Mix the latex suspension thoroughly by shaking vigorously through three or four times then let stand for 15 to 20 minutes before reading. 5. Mix the latex suspension thoroughly by shaking vigorously through three or four times then let stand for 15 to 20 minutes before reading.

The resting level of approximately 5 µg/ml in healthy persons, exceptionally high levels have been demonstrated in pulmonary disease, with a haemagglutination-inhibition immunoassay 13,14,15. Thrombo-Wellcotest are extremely clear cut and can easily be recognised under any normal conditions of lighting. Granularity may be detected in negative patterns, depending on the visual acuity of the operator. Due to a difference in the liquid properties of serum and urine, the volume of one drop of serum is less than the volume of one drop of urine as dispensed by the pipettes provided with the sample kit. The amounts for preparing for the 1/5 dilutions of serum and urine are not identical. Semi-quantitative Procedure for Serum Samples 1. Prepare a 1/20 dilution of the serum sample by adding 0.1 ml of serum to 0.9 ml of saline buffer in a test tube. 2. Prepare a doubling dilution series from 1/10 to 1/120, or as specified. 3. Transfer 1 drop of each dilution starting from 1/320 to separate positions on the glass slide, using disposable pipette and built-up. 4. Mix the latex suspension thoroughly by shaking vigorously through three or four times then let stand for 15 to 20 minutes before reading. 5. Mix the latex suspension thoroughly by shaking vigorously through three or four times then let stand for 15 to 20 minutes before reading. 6. Rotate the slide slowly for exactly two minutes while looking for obvious agglutination. 7. The end point is the last serum dilution which shows obvious agglutination.
Sample therefore contains at least 2 µg/ml when diluted 1/20, therefore the FDP level is 40 µg/ml or greater.

Sample 2 – Urine

Pattern undiluted (ring 1) – positive. Pattern diluted 1/5 (ring 2) – negative. Urine undiluted contains 2 µg/ml or greater but less than 2 µg/ml when diluted 1/25. FDP level in urine is therefore between 2 and 10 µg/ml.

Semi-quantitative Results

Calculation of Results

Serum and urine FDP levels are calculated identically by multiplying the dilution at the end point of the titration by a factor of 2 µg/ml. e.g. serum

FDP level in serum is 20 x 2 = 40 µg/ml (approx).

QUALITY CONTROL

The reagents have good stability and it is not necessary to include known positive or negative control sera in each batch of tests. When an internal standard of several days has occurred since the last test, however, it is wise to take precaution to ensure the proper functioning of the system by testing the latex suspension with the control sera provided with the kit. These have been diluted ready for use: place one droplet of each on the slide and proceed with the test as from Step 6 of the Qualitative Procedure. The positive control should show agglutination while the negative control should not. If greater accuracy is required in the semi-quantitative procedure, it is recommended that a suitable reference preparation be incorporated in each assay.

INTERPRETATION OF RESULTS

FDP levels measured using Thrombo-Wellcotest correlate well (r = 0.95) with results obtained with haemagglutination inhibition immunofluorescence. Samples investigated include dermatomyositis (28), sera from patients with renal disease, liver disease, carcinoma, hyperthyroidism, pulmonary embolism, thromboembolic and myocardial infarction (1); and urine from normal individuals and patients with recent renal transplants.

The interpretation of results obtained with Thrombo-Wellcotest is therefore the same as for those obtained using HIV techniques.

Serum FDP Levels

Since the mean normal level of serum FDP is 9 ± 2 µg/ml (normal or slightly elevated samples will give negative [non-agglutinated] results in addition to values when tested by the method described. Although moderately raised levels have been found in a wide variety of disease states, the normal serum level recorded for FDP may be of diagnostic value in disseminated intravascular coagulation (DIC). &FDP estimation may provide useful clinical information on the type, activity and severity of the disease.

FDP levels may give early warning of an extension of the infarct or secondary complications subsequently observed. In patients with acute occlusive vascular diseases which are difficult to detect, as for example, pulmonary embolism is associated with marked elevation of FDP, levels fall rapidly when effective immunosuppressive treatment is resumed. In cases of urinary tract infection the assay of urinary FDP can help in the diagnosis of the site of infection; raised levels being associated with upper tract infection while patients with bladder infections show normal levels.

Urine FDP Levels

Normal urine contains less than 0.25 µg/ml FDP, giving negative (non-agglutinated) patterns in both positions when tested and described above. In patients with kidney disease, increased levels may be found and there is evidence that a daily serum or urinary FDP estimation may provide useful clinical information on the type, activity and severity of the disease. The effectiveness of certain drugs in the treatment of proliferative glomerulonephritis can be monitored by following the pattern of FDP excretion over the period of administration. Similarly, during resection cases, following renal transplantation FDP levels may rise rapidly to levels as high as 75 µg/ml. By monitoring urinary FDP, incipient rejection may be detected before it becomes apparent in other ways, levels fall rapidly when effective immunosuppressive treatment is resumed. In cases of urinary tract infection the assay of urinary FDP can help in the diagnosis of the site of infection; raised levels being associated with upper tract infection while patients with bladder infections show normal levels.

b. LIMITATIONS OF PROCEDE

With serum samples, the presence of rheumatoid factor may interfere with the test and cause falsely high results. If a patient is suspected of having rheumatoid arthritis, it is advisable to run a test for rheumatoid factor in parallel with Thrombo-Wellcotest. If this test gives a negative result, the FDP level can be read as valid; however, with both tests positive, the FDP level should be interpreted with caution.

Rheumatoid factor interference may be eliminated by reduction of the serum with dithiothreitol or 2-mercaptoethanol. Certain substances that may be present in urine can cause non-specific agglutination. These interfering substances can be removed as described under Supplement Collection – Urine Samples.

c. EXPECTED RESULTS

Thrombo-Wellcotest latex will agglutinate in the presence of FDP at a concentration of 2 µg/ml or greater.

20. SPECIFIC PERFORMANCE CHARACTERISTICS

Thrombo-Wellcotest is capable of detecting fibrinogen and all related antigens (fibrin monomer, fragments X, Y, D and E) in human plasma, serum and urine. The sensitivity of the test is standardised at 2 µg/ml FDP but the reactivity toward the intact fibrinogen molecule may vary from batch to batch. It is recommended if Thrombo-Wellcotest is used for any purpose other than the measurement of FDP in serum or urine as described in the Instructions for Use, a laboratory standard preparation of an appropriate material should be included with each batch of tests.

REFERENCES


