INDIRECT LATEX SLIDE TEST FOR DETECTION OF MICROALBUMINURIA

SUMMARY
Urinary albumin excretion between 30-300 mg/day (Microalbuminuria), far below the levels found in clinical proteinuria (> 300 mg/day) is a strong predictor of development of Diabetic nephropathy and vascular complications. These low levels of albumin excretion are detectable only by sensitive immunoassays for microalbuminuria. Diabetic nephropathy leads to progressive loss of renal function or end stage renal disease (ESRD) and may necessitate need for dialysis or transplantation in most cases. The progression of microalbuminuria is closely associated with progressive hypertension and loss of blood glucose control. The early presence of microalbuminuria can be reversed by strict metabolic control and timely intervention of drugs early in the course of disease can arrest the progression of diabetic renal disease. Annual screening of microalbuminuria is recommended by W.H.O. and International Diabetes Foundation in all patients with insulin dependent diabetes mellitus (IDDM) over the age of 12 years and who have had diabetes for five years or more. Microalbuminuria is also a significant risk marker of cardiovascular diseases. Its presence can be regarded as an index of increased cardiovascular vulnerability and a signal for correction of known risk factors. MICROTEX is a sensitive immunoassay useful for the detection of microalbuminuria.

PRESENTATION

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>VIAL VOLUME</th>
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</thead>
<tbody>
<tr>
<td>R1</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>R2</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>CONTROL +VE</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>CONTROL -VE</td>
<td>0.4 ml</td>
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</tbody>
</table>

REAGENT
1. Anti-human Albumin reagent (R1): The concentration of the Agglutinating sera to human albumin is adjusted to provide sensitivity of about 25 mg/L and above of Microalbuminuria.
2. Albumin Latex reagent (R2): A uniform suspension of polystyrene latex particles to which human albumin has been chemically coupled.
   Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity and performance.

REAGENT STORAGE AND STABILITY
Store the reagents at 2-8°C. DO NOT FREEZE. The shelf life of the reagents is as per the expiry date mentioned on the reagent vial labels.

PRINCIPLE
MICROTEX slide test for the detection of Microalbuminuria is based on the principle of agglutination inhibition. The urine specimen to be tested is first mixed with the Agglutinating sera directed against human albumin. The latex coupled with human albumin is added to the mixture and is allowed to react. When the urine specimen does not contain albumin, antibodies to human albumin would be free to react with the latex coupled with human albumin causing agglutination. When the urine specimen contains at least 25 mg/L of albumin, the Agglutinating sera to human albumin will be neutralized and will not be available to react with latex coupled with human albumin. Hence no agglutination will be observed.

NOTE
1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and found to be non-reactive. However handle the material as if infectious.
3. The reagents contain 0.1% sodium azide as preservative. Avoid contact with skin or mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of reagents should be verified with positive and negative controls provided with the kit.
5. Use reagents of same lot numbers. Do not interchange reagents of different lot numbers.
6. Do not interchange reagent vial droppers.
7. Shake the Albumin latex reagent (R2) vial well before use to disperse the latex particles uniformly and improve test readability.
8. Only a clean and dry glass slide must be used. Slide should be free from even traces of protein compounds.
9. Accessories provided with the kit only must be used for optimum results.
10. Do not use damaged or leaking reagents.

SAMPLE COLLECTION AND PREPARATION
For Qualitative Method
Though random urine specimens can be used, first morning urine specimen is preferable. Specimens should be collected in clean glass or plastic containers free of detergents. Specimens should be tested immediately preferably within 12 hours of collection. Should a delay in testing occur add thimerosal (0.01%) or sodium azide (0.1%) to the specimen and store at 2-8°C up to 72 hours. Do not use grossly contaminated specimens. If specimen is cloudy or contains blood, centrifuge the specimen at 1000 rpm for one minute and use clear supernatant for testing.

For Semi Quantitative Method
Urine specimens collected over a 24 hour period should be pooled in a clean detergent free container and refrigerated at 2-8°C. Thimerosal (0.01%) or sodium azide (0.1%) are recommended as urine preservatives.

MATERIALS PROVIDED WITH THE KIT
Reagents
Anti-human Albumin reagent (R1), Albumin Latex reagent (R2), Positive control reactive with Anti-human albumin reagent, Negative control nonreactive with Anti-human albumin reagent.

Accessories
Glass slide with six reaction circles, Pipettes for dispensing urine specimen, Mixing sticks and Rubber teats.

ADDITIONAL MATERIAL REQUIRED
A high intensity direct light source, stopwatch.

TEST PROCEDURE
Bring all reagents and samples to room temperature before use.
Qualitative Method
1. Place one drop (50 µl) of clear urine under test on the glass slide using disposable pipettes provided with the kit. Deliver the drop vertically.
2. Add one drop of Anti-human albumin reagent to the drop of urine under test on the slide. Deliver the drop vertically. Do not let the dropper tip touch the liquid on the slide.
3. Using a mixing stick, mix the Anti human albumin reagent and urine over the circle for 30 seconds.
4. Add one drop of well-mixed Albumin latex reagent to the mixture. Do not let the dropper tip touch the liquid on the slide. Mix uniformly over the entire circle.
5. Immediately start the stopwatch, rock the slide gently back and forth observing for agglutination macroscopically at three minutes.

Semi Quantitative Method
Measure and record the total volume of patient urine collected over a 24-hour period. Centrifuge an aliquot of the 24-hour urine specimen. Using isotonic saline prepare progressive dilutions from the centrifuged urine specimen. Perform the qualitative test procedure using each dilution as specimen.

INTERPRETATION OF RESULTS
Qualitative Method
Agglutination is a negative test result indicating the absence of detectable levels of albumin in urine signifying absence of Microalbuminuria. No agglutination is a positive test result indicating the presence of albumin in concentrations above 25 mg/L in urine signifying Microalbuminuria.

Semi Quantitative Method
No agglutination in the highest urine dilution corresponds to the titre of microalbumin per litre of the specimen. To calculate the concentration of microalbumin in the specimen use the following formula:

Microalbumin (mg/L) = S X D

where,  S = Sensitivity of the test i.e. 25 mg/L
       D = Highest dilution of urine showing no agglutination.

REMARKS
1. Microalbuminuria also occurs in response to acute inflammatory conditions such as Ischaemia, trauma and thermal injury, surgery, pancreatitis and inflammatory bowel disease. In many of these conditions the albumin excretion increases within minutes or hours of the initiating stimulus and only lasts for 24-72 hours.
2. Use only urine as test specimen. Do not use serum.
3. Albumin excretion is increased after physical activity. It is therefore recommended to use urine sample that has been produced at rest whenever random urine specimen is used.
4. As albumin excretion is subject to physiological fluctuations it is necessary to take two measurements in consecutive days, in case of contradictory results three measurements on different days must be done preferably within a week.
5. Liquid intake of the patient must be in the normal range i.e. 1.5-2 litres/day.
6. To diagnose incipient nephropathy microalbuminuria must be present in at least 2 out of 3 specimens over a 3-6 month period.
7. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
8. It is recommended that reagents should be tested with positive and negative controls periodically to validate their performance.

PERFORMANCE CHARACTERISTICS
The performance of Microtex was evaluated using 15 albumin positive and 40 albumin negative urine samples. The urine samples were validated using Quantia®MA, an immunoturbidimetric assay for detection of microalbumin in urine.

<table>
<thead>
<tr>
<th>Specimen data</th>
<th>No. of samples</th>
<th>Microtex</th>
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<tbody>
<tr>
<td></td>
<td>Albumin + VE</td>
<td>Albumin - VE</td>
<td></td>
</tr>
<tr>
<td>Albumin + VE samples</td>
<td>15</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Albumin Negative samples</td>
<td>40</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>15</td>
<td>40</td>
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Sensitivity: 100%  Specificity: 100%

WARRANTY
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty to use and sale for any other purpose.

BIBLIOGRAPHY
7. Data on file: Tulip Diagnostics (P) Ltd.