IMMUNNOTURBIDIMETRIC ASSAY FOR DETERMINATION OF SERUM CYSTATIN C ON TURBODYNE™ SC

SUMMARY
Cystatin C, a non glycosylated, low molecular weight (13kDa) protein belongs to the family of cysteine protease inhibitors. Cystatin C is produced at a constant rate by nearly every nucleated cell in the human body. Its biochemical characteristics allow its free filtration in the glomerulus. Subsequently it is reabsorbed and almost completely catabolized in the proximal tubule. Practically no Cystatin C returns to the blood. Therefore Cystatin C concentration in human blood is closely related to Glomerular filtration rate (GFR). An increased Cystatin C concentration in human blood may indicate a reduced GFR, which may be due to renal diseases. The production of Cystatin C in the body is not influenced by renal condition, increased protein catabolism or dietetic factors. Moreover, it does not change with age or muscle mass like creatinine does. Serum Cystatin C is therefore proposed to be a ideal endogenous marker of glomerular filtration rate (GFR) especially in patients with moderate to severe renal impairment. Studies have demonstrated that Cystatin C is the most suitable marker of moderately impaired renal function.

Turbodyne™ Cystatin C is an Immunoturbidimetric assay useful for quantitative measurement of Cystatin C in human serum/plasma.

PRESENTATION

<table>
<thead>
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<th>REF</th>
<th>108510020</th>
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<tbody>
<tr>
<td>v</td>
<td>20 Tests</td>
<td>60 Tests</td>
</tr>
<tr>
<td>R1</td>
<td>4.8 ml</td>
<td>3 x 4.8 ml</td>
</tr>
<tr>
<td>R2</td>
<td>1.2 ml</td>
<td>3 x 1.2 ml</td>
</tr>
<tr>
<td>SC</td>
<td>1 No.</td>
<td>1 No.</td>
</tr>
<tr>
<td>CT</td>
<td>20 Nos.</td>
<td>60 Nos.</td>
</tr>
</tbody>
</table>

REAGENT

1. **Turbodyne™ Cystatin C Activation Buffer (R1):** Ready to use buffer solution.
2. **Turbodyne™ Cystatin C Latex Reagent (R2):** Purified immunoglobulin fraction that is directed against Cystatin C which is covalently linked to uniform suspension of polystyrene latex particles.
3. **Turbodyne™ Cystatin C Smart card:** Card with Cystatin C Master calibration Curve with a standard that has been validated against the IFCC standard.

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagents is as per the expiry date mentioned on the respective vial labels.
3. Once opened the reagents are stable for 75 days when stored at 2-8°C provided the reagents are not contaminated.
4. Store the Turbodyne™ SC Smart card at a clean dry place. The Turbodyne™ SC smart card is valid upto the use of labelled number of tests within 75 days from its first insertion in the Turbodyne™ SC analyzer.

PRINCIPLE

Turbodyne™ Cystatin C is a turbidimetric immunoassay for the quantitative determination of Cystatin C in human serum and is based on the principle of agglutination reaction. The test specimen is mixed with Cystatin C latex reagent (R2) and activation buffer (R1) and allowed to react. Presence of Cystatin C in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at wavelength of ~ 650 nm. The extent of turbidity corresponds to the concentration of Cystatin C in the specimen.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. All the reagents derived from human source have been tested for HBsAg and HIV antibodies and are found to be non reactive. However handle the material as if infectious.
3. Reagent contains 0.09% Sodium Azide as preservative in concentrations that is not characterized as dangerous. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. Gently mix the Turbodyne™ Cystatin C latex reagent (R2) well before use to disperse the latex particles uniformly to improve test performance.
5. As the reagents and Smart card within lots have been matched, reagents or Smart cards from different lots must not be interchanged.

6. It is recommended that the performance of the reagent and Smart Card calibration must be validated periodically with known controls such as Turbodyne™ Cystatin C (Ref: 108590002).

7. Do not use damaged or leaking reagents.

8. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures.

9. Always use clean disposable micropipette tips to aspirate the reagents to prevent contamination.

SPECIMEN COLLECTION AND PREPARATION
No special preparation of the patient is required prior to specimen collection by approved techniques.

EDTA/Heparinized plasma or serum should be used for testing. Store the samples at 2 - 8°C. Samples can be stored for up to one week at 2 - 8°C, provided they are not contaminated. Do not use hemolysed, icteric, or highly turbid sera. Turbid or particulate samples must be clarified by centrifugation at 2000 rpm for 15 minutes prior to testing. Use the clear supernatant for testing.

ADDITIONAL MATERIAL REQUIRED
Turbodyne™ SC analyser, stopwatch, well calibrated micropipettes, disposable tips, incubator.

TEST PROCEDURE
1. Bring reagent and sample to room temperature before use.

2. Insert the Turbodyne™ Cystatin C smart card reader in the card reader slot of the Turbodyne™ SC as described in the user manual.

3. The instrument will indicate to place cuvette with R1 + sample in the reading chamber.

4. Take a Turbodyne™ cuvette and add 240 µl R1 to the cuvette. Then add 5 µl sample and incubate the cuvette for 5 minutes.

5. Place the cuvette with R1 + sample in the Turbodyne™ SC reading chamber.

6. Press “Testing”. The instrument will mix the sample and then indicate to add R2.

7. Pipette 60 µl R2 reagent with the Turbodyne™ SC electronic Pipette to the cuvette with R1+sample.

8. The reaction will start and the counter will start in the display. Results will be displayed on completion of reaction.

For GFR prediction calculation:
For calculation of GFR from Cystatin C values measured with Turbodyne™ Cystatin C assay the following prediction equation is recommended using mg/L as the unit factor.

\[
\text{GFR} \left[ \frac{\text{mL/min}}{1.73\text{m}^2} \right] = \frac{79.901}{1.4389} \times \text{Cystatin C (mg/L)}
\]

GFR can be calculated with the GFR calculator available on our website www.tulipgroup.com.

Quality control
The calibration of Turbodyne™ Cystatin C must be validated with Turbodyne™ Cystatin C Control set.

SPECIFIC PERFORMANCE CHARACTERISTICS
Measuring Range
The Turbodyne™ Cystatin C assay has been designed to measure Cystatin C concentration in the range of 0.5 – 8.0 mg/L. The exact range is dependant on the calibrator value used for calibration which is lot specific. The Turbodyne™ Cystatin C assay is linear within the measuring range.

Detection limit / Analytical Sensitivity
0.5 mg/L. The detection limit represents the lowest measurable Cystatin C concentration that can be distinguished from zero.

Precision

<table>
<thead>
<tr>
<th>Intra-assay precision</th>
<th>n</th>
<th>Mean mg/L</th>
<th>SD</th>
<th>CV (%)</th>
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</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>10</td>
<td>2.0</td>
<td>0.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Sample 2</td>
<td>10</td>
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<td>Sample 3</td>
<td>10</td>
<td>5.0</td>
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<td>5.3</td>
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<tr>
<td>Sample 2</td>
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<td>4.6</td>
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<tr>
<td>Sample 3</td>
<td>10</td>
<td>4.9</td>
<td>0.2</td>
<td>3.8</td>
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</table>

Interference
No interference was observed with Hemoglobin 8 g/L, Bilirubin 420 mg/L, and Triglycerides 12.5 mmol/mL. Interference of RF does not take place with Turbodyne™ Cystatin C assay as it uses avian antibodies.
REFERENCE VALUES
The reference values for Cystatin C was determined to be <1.16 mg/L.
It is recommended that each laboratory must define its own reference range for relevant population taking into account all affecting factors.

REMARKS
1. Usage of well-calibrated pipette and correct procedure is critical for achieving correct results.
2. Markedly lipemic, hemolysed, and contaminated serum samples could produce non-specific values.
3. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.
4. Several Cystatin C based prediction equations for calculation of GFR for adults and children have been published. It should be noted that these formulas were evaluated with different Cystatin C assays and may reveal inaccurate GFR.
5. In contrast to creatinine concentration, Cystatin C levels are lower in hypothyroid and higher in hyperthyroid state as compared with the euthyroid state. Therefore thyroid function has to be considered when Cystatin C is used as a marker of kidney function.
6. Samples with values above measuring range must be diluted 1:4 with normal saline and retested. The result obtained must be multiplied with the dilution factor.

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

BIBLIOGRAPHY
2. Patricia Villa et.al., Serum Cystatin C as a marker of acute renal dysfunction in critically ill patients. Critical Care 2005, 9, pgs R139 - R143.
7. Data on file: Tulip Diagnostics (P) Ltd.
<table>
<thead>
<tr>
<th>SYMBOL KEYS</th>
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**TULIP DIAGNOSTICS (P) LTD.**

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