Quantitative determination of Lambda Light Chain in human serum or in urine. Turbidimetric method.

**METHOD AND PRINCIPLE**
Quantitative turbidimetric test for the measurement of total Lambda light chains in human serum (total chains = bound + free) or free chains in the urine (free chains). Human Lambda Light Chains antibodies form insoluble complexes when mixed with samples containing light Lambda chains giving turbidity to the solution. The developed turbidity depends on the concentration of the light Lambda chains in the patient sample and can be measured photometrically at 340 nm. The use of a calibration curve allows to quantize the light Lambda chains present in the sample.

**CLINICAL SIGNIFICANCE**
Immunoglobulin molecules (antibodies) are proteins composed of two identical "heavy" peptide chains joined by two identical "light" chains. There are five different types of heavy chains (which determine the class of immunoglobulin, IgG, IgA, IgM, IgE, IgD) but only two types of light chains, K and \( \lambda \) (Lambda).

The ability to recognize and bind the antigen is due to the terminal part (variable region) of both heavy and light chains. Normally, a small amount of light chains in excess of heavy ones is produced by the lymphocytes of the immune system: these chains that do not combine to form complete immunoglobulins are released into the blood and allowed to pass in small quantities by the kidneys in the urine.

The increase in blood concentration of the light chains K or \( \lambda \) (Lambda) can be of two types: polyclonal or monoclonal (Bence Jones Protein). Polyclonality means that the light chains present are those produced by all the sets (clones) of lymphocytes that produce all the antibody repertoire, a specific defense mechanism of the organism. An increase in polyclonal light chains may be due to renal damage, which diminishes their ability to metabolise and eliminate them: both types K and \( \lambda \) (Lambda) increase, so their relationship remains unchanged. On the contrary, an increase in the concentration of monoclonal light chains derives from an excess of production of chains of a single type by a single clone of lymphocytes, which proliferates beyond its normal limits (as in the case of multiple myeloma, Waldenstrom macroglobulinemia, non-Hodgkin lymphoma, etc.).

The quantitative determination of the presence of the K and \( \lambda \) (Lambda) chains in the serum and in the urine, as well as the diagnostic value, has great importance in monitoring of the therapy.

The clinical indication of this assay is the diagnostic deepening in the presence of a monoclonal component detected by electrophoresis; coupled to the search for \( \lambda \) (Lambda) chains (K/kappa) / \( \lambda \) (Lambda) ratio, it can be used for the screening of relevant monoclonal gammopathies.

**REAGENT COMPOSITION**

**Reagent (R1):**
- Tris Buffer, pH 7.5: 100 mmol/L
- Sodium azide: 0.1%

**Reagent (R2):**
- Tris Buffer, pH 7.5: 100 mmol/L
- Anti-human Lambda Light Chain Antibody (Goat)

**REAGENT PREPARATION AND STABILITY**
Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Absorbance of Blank Reagent >0.300 at 340 nm in cuvette 1 cm against water. After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

**SPECIMEN**

**Serum:**
Use fresh serum. If the test can not be performed on the same day, the serum can be stored at 2-8 °C for 48 hours. If it is necessary to store it for a longer period, the sample must be frozen. Centrifuge the samples containing precipitate before performing the test. Fibrin samples should be centrifuged before testing. Avoid hemolysate or lipemic samples. Separate the serum from the clot quickly. Perform a single defrost.

**Urine:**
The collection of the 24-hour urine sample involves acidification of the urine itself. Acidification is carried out by pouring into the container, before starting the collection, about 5 mL of 5 M HCl (available at the laboratory where the sample is taken) for each liter of urine or 4 tablespoons of muriatic acid of the trade. Take an aliquot of approximately 10 mL of acidified 24 hours urine, record the total diuresis and deliver to the laboratory.

**PROCEDURE**

**Wavelength:** 340 nm
**Temperature:** +37°C
**Measurement** against distilled water

<table>
<thead>
<tr>
<th>Reagent (R1)</th>
<th>Serum procedure</th>
<th>Urine procedure</th>
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<tbody>
<tr>
<td>350 µL</td>
<td>250 µL</td>
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</table>

Sample / Calibrator/H2O 2 µL 8 µL

Mix and after 120° read Absorbance (Abs1). Then add:

Reagent (R2) 75 µL 30 µL

Mix, after other 300° read Absorbance again (Abs 2). Calculate \( \Delta \) Abs (Abs 2 – Abs 1) for samples and calibrators.

**CALIBRATION**
The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

**Serum Calibration:**

*Use MTD Diagnostics product:*

- Plasma Protein Multicalibrator
- REF TUC1030 (3x1 mL)
Lambda Light Chain Turbidimetric

Dilute in NaCl 9g/L as follows:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAL (µL)</td>
<td></td>
<td>10</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>NaCl 9 g/L (µL)</td>
<td>100</td>
<td>90</td>
<td>75</td>
<td>50</td>
<td>25</td>
<td>--</td>
</tr>
<tr>
<td>Factor</td>
<td>0.0</td>
<td>0.1</td>
<td>0.25</td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Multiply the concentration of Calibrator by the corresponding factor to obtain the concentration of each calibrator dilution.

To avoid to dilute the Multicalibrator, it is possible to use:

- **Plasma Protein Multicalibrator Set**: REF TUC1035 (5x1 mL)
- **Plasma Protein Control Level 1**: REF TUC1036 (2x5 mL)
- **Kappa / Lambda Urine Calibrator**: REF TUC1037 (1x1 mL)

Dilute using the same table indicated above for the serum.

**CALCULATION**

The analytical session cannot be validated if the Δ Abs (Abs2 - Abs1) of the Blank Reagent is > 0.300 at 340 nm in a 1 cm cuvette of optical path. Plot the different ΔAbs (Abs2-Abs1) absorbances against the concentration of each calibrator dilution. The concentration of the sample is calculated by interpolation of its ΔAbs (Abs2-Abs1) value on the calibration curve. For automatic calculation, use the SPLINE curve but other mathematical method can be used (Point-Point; Logit-Log 4P, etc.).

Conversion Factor: mg/dL x 0.01 = g/L ; mg/L x 0.1 = mg/dL

**QUALITY CONTROL**

Normal and abnormal control sera/urine of known concentration should be analysed routinely with each group of unknown samples utilizing MTD Diagnostics Quality Control Material:

- Plasma Protein Control Level 1 - REF: TUC1040 (3 x 1 mL)
- Plasma Protein Control Level 2 - REF: TUC1050 (3 x 1 mL)
- Kappa / Lambda Urine Control - REF TUC1038 (1x1 mL)

The range of the values of the controls must be evaluated as a guideline, since it can be determined by the application of the method or by the user’s manual skills or by other factors. The values obtained must be used for the evaluation of the Precision of the method (Repeatability). For the evaluation of the Accuracy of the method (Reproducibility) it is necessary to adhere to a program of External Quality Assessment (EQA) managed by certified bodies.

**EXPECTED VALUES**

- **Serum**: 110 – 240 mg/dL (IFCC) (total chains = bound + free)
- **Urine**: < 1 mg/dL (10 mg/L) (free chains)

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient’s medical history, clinical examination and other results.

**PERFORMANCE**

**PRECISION:**
- Low Level: Serum Samples (n) = 20 : Mean 115 – SD 2.53 – CV% 2.2
- High Level: Serum Samples (n) = 20 : Mean 209 – SD 4.32 – CV% 2.06
- Urine Samples (n) = 20 : Mean 25 – SD 0.61 – CV% 2.41

**ACCURACY (CORRELATION):**

A comparison between MTD Diagnostics method (y) and a commercially available test (x) gave following results:

- Serum : y = 1.002 x – 0.71 \( r = 0.999 \)
- Urine : y = 1.18 x – 0.43 \( r = 0.998 \)

**LINEARITY:**
- Serum 20 – 450 mg/dL ; Urine 1-20 mg/dL

**SENSITIVITY:**
- Serum 20 mg/dL ; Urine 1 mg/dL

**SPECIFICITY / INTERFERENCES**

No interferences was observed by Bilirubin up to 20 mg/dL, Hemoglobin up to 1000 mg/dL, Triglycerides up to 800 mg/dL. Other substances may interfere.

**PRECAUTIONS**

R1 and R2 contain TRIS BUFFER 100 mmol/L – pH 7.5 - CAS 1185-53-1

H315: Causes skin irritation

H319: Causes serious eye damage

H335: May cause respiratory irritation

The antibody present in the preparation are of animal origin and are not capable of transmitting infectious diseases to humans. However, since there are no methods to ensure the total absence of such infectious agents or of other microbes, this product must be handled as if it were risky and potentially capable of transmitting infectious diseases of any kind, in accordance with Good Laboratory Practice standards.

The products do not contain other dangerous substances or mixtures, according to the EC Regulation n° 1272/2008 or their concentrations are such as not to be considered persistent, bioaccumulative or toxic (PBT). The product is classified and labeled in accordance with EC directives or respective national laws. Sodium azide, less than 0.1%.

However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:

- S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.
- S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.
- S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.
- S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.
- S63 (P304 – P340): In case of accidental injury: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

**FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.**

**SIMBOLOGY**

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<tr>
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**BIBLIOGRAPHY**