TRIMERO Diagnostics, SL
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CE IVD

EN

INSTRUCTIONS FOR USE

Reagents for professional use,
for *In Vitro* use only in clinical laboratory (IVD)

3diag - U-A1m - TIA

Alpha-1 Microglobulin - Urine for Turbidimetry

REF **TD-42831**

INTENDED USE

Quantitative determination of Alpha-1 Microglobulin (A1m), in human urine, by turbidimetric method in automatic Clinical Chemistry Analyzers.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, bound to polystyrene particles, when combined with the antigens (Ag) of the patient sample, form insoluble compounds causing a change in the absorbance and dispersion of the light, proportional to the antigen concentration, which can be quantified by turbidimetric (TIA) or nephelometric (NIA) method, by comparison with calibrators of known concentration.

CONTENTS - COMPOSITION - PREPARATION

- Antiserum Reagent: **REAG | Ab | U-A1m**
  REF TD-42831-RA ▽ 100 test (*1) - 9 ml
  Anti-human A1m antibodies bound to polystyrene particles.
- Reaction Buffer: **BUF | U-A1m**
  REF TD-42831-BF ▽ 100 test (*1) - 9 ml
  TRIS Buffer, with PEG.

Note (*1): with the recommended general assay parameters.

As a preservative, the reagents contain <0.1% (1 g/l) Sodium Azide (NaN_3).

The reagents are ready for use and require no preparation.

Before each use it is convenient that the reagents are homogenized, shaking them gently avoiding the formation of foam or bubbles.

WARNINGS - PRECAUTIONS

- Sodium Azide is toxic. Even if sodium azide is not harmful at the concentration present in the reagents, take the necessary precautions to avoid accidental ingestion or contact with the eyes.
- Sodium Azide can react with lead or copper to give explosive compounds. For disposal it is recommended to rinse with plenty of running water to avoid accumulation in drains.
- Since the absence of infectious agents can not be proven with absolute certainty, components containing materials of human or animal origin must be handled with caution, as potentially infectious, following the recommended safety standards for biological risk.
- Do not mix components belonging to different lot kits.
- Clinical diagnosis should not be based on the results of a single test, but should always integrate all relevant clinical and laboratory data.

STORAGE - SHELF LIFE

- Store refrigerated at +2...+8ºC. Do not freeze, as the functionality of the reagents may be altered.
- Properly stored and unopened, the reagents are stable until the expiration date indicated on the label.
- Once opened, the shelf life of the reagents is at least 4 weeks, provided that after each use they are stored immediately in the original containers, tightly capped and refrigerated at +2...+8ºC. This information should be taken as a guideline given that, obviously, the shelf life depends on the particular environmental and use conditions, which may differ from those of the stability studies carried out.

MATERIALS NEEDED, NOT SUPPLIED

- Automatic Clinical Chemistry Analyzer, capable of running photometric assays at 600...660 nm, and accessories: reagent containers, cuvettes, etc..
- 3diag - U-A1m - CAL SET — REF TD-42832
- 3diag - U-A1m - CONTROL — REF TD-42833

SAMPLES

Fresh urine.

It is usual the use of a 24-hour urine aliquot, although in the literature some authors recommend the use of random urine, preferably the second morning void, and expressing the results relative to urinary creatinine.

Prior to the analysis, the samples should be centrifuged until a clear and transparent supernatant is obtained[1].

For the determination of specific proteins, centrifugation of urine samples at 3000[2]-5000[3] g for 10 minutes is the standard practice in the laboratory.

In bibliography[4] it is reported a stability of 7 days in refrigerated urine (sample of preference).

PROCEDURE

If necessary, carefully transfer the reagents to the containers used by the analyzer, preventing leakage and foaming or bubbles.

To program and calibrate assays, follow the instructions for use of the analyzer used, with the recommended general parameters that are detailed below. Please, contact the Customer Support Service (✉ support@3diag.com - ☎ +34 93 244 86 79) for further information about applications to specific analyzers.

Assay Parameters

- ①Dispense and mix:
  - Sample/Calibrator/Control: 2 µl (neat)
  - **BUF | U-A1m** 90 µl
- ②Incubate a fixed time between 1 and 5 minutes
- ③Dispense and mix:
  - **REAG | Ab | U-A1m** 90 µl
- ④Read absorbance A1 (Blank) at 600...660 nm
- ⑤Incubate a fixed time of between 3 and 5 minutes
- ⑥Read absorbance A2 (Final Point) at 600...660 nm
- ⑦Interpolate the absorbance increment (A2-A1) of the samples and controls in the curve obtained with the calibrators
- ⑧Samples with concentrations higher than the upper limit of the assay range should be analyzed again, diluted manually with Physiological Solution, or by programming a larger sample dilution in the analyzer, to recover a value close to the midpoint of the measurement range.

As an alternative, reagents can be mixed as first step, and the sample dispensed as starter.

Calibration Parameters

- Use the 3diag - U-A1m - CAL SET.
- If the analyzer allows it, it is recommended to program two replicates of each calibration point.
- The calibrations are Non-linear. For the calculation it is recommended to use a 3rd Order Polynomial, a Logit or a Polygonal adjustment.
- The assay must be recalibrated, at least when a new batch of reagents is used or when its parameterization is changed.

PERFORMANCES OF THE METHOD

Detailed information on the characteristics and performances of the assay is given in the Technical Report, available on the website (www.3diag.com) or upon request to the Customer Support Service (✉ support@3diag.com - ☎ +34 93 244 86 79).

QUALITY CONTROL

To monitor performances, it is recommended that internal controls be inserted into each analytical series. It is recommended to use the controls of **3diag - U-A1m - CONTROL**.

Each laboratory should establish its own quality scheme and corrective actions if controls do not meet the assigned tolerances.

The reagents have been subjected to quality control checks and should react as described in these instructions. Therefore, as a general recommendation, in case the controls do not give the expected reaction, as a precaution all reagents should be considered unreliable until their operation has been checked.

TRACEABILITY

Given that certified reference materials are not available, values are referred to internal standards based on highly purified proteins.

REFERENCE INTERVALS

It is always advisable for each laboratory to establish its own reference values.

The bibliography[4] reports the following reference values:

- Adults (>16 years): up to 19 mg/24h
- Adults (<50 years): up to 13 mg/g-Creatinine
- Adults (>50 years): up to 20 mg/g-Creatinine
- Children (<16 years): up to 7 mg/g-Creatinine

CLINICAL SIGNIFICANCE

Alpha-1 Microglobulin (A1m) is a low molecular weight glycoprotein (26 kDa), stable at altered pH. It is synthesized in the liver, is freely filtered by the glomeruli, and is reabsorbed by the proximal tubules, where it is catabolized.

Under normal conditions, a very little amount of A1m is excreted in the final urine. Therefore, an increase in urinary concentration is indicative of lesions of the proximal tubule and/or tubular dysfunction, due to any cause or pathology.

SYMBOLS

In addition to the harmonized symbols provided on the European Standard EN 980:2008, in the labels and instructions of use has been used the complementary symbology proposed[5] by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

REAG	Reagent
Ab	Antibody / Antiserum
BUF	Buffer
U-A1m	Alpha-1 Microglobulin - Urine

BIBLIOGRAPHY

(1) Morales LJ., Ventura S., Solé E et al. - Comite de Comunicación de la Sociedad Española de Medicina de Laboratorio, SEQC^{ML}: "*Muestras de Orina de 24 horas y Orina Reciente para la Medición de las Magnitudes Biológicas Más Comunes*", ISBN: 978-84-89975-52-1 (2017).

(2) "*Alpha-1-Microglobulin (A1M) - IMMAGE® Immunochemistry Systems Chemistry Information Sheet*", © Copyright 2017 Beckman Coulter, Inc..

(3) Bergón Jiménez E., Bergón Sendín M.: "*Uso del cociente cadenas kappa/cadenas lambda en orina para el estudio de la proteína de Bence Jones*", Química Clínica 1999; 18 (5) 266-270.

(4) *Mayo Medical Laboratories* website (www.mayomedicallaboratories.com), date of consultation: 25th October 2017.

(5) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

TEXT REVISION DATE

27th July 2020.

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INSTRUCTIONS FOR USE

Reagents for professional use,
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3diag - U-A1m - CAL SET

Alpha-1 Microglobulin - Urine
Calibrators (2 lev.)
REF **TD-42832**

INTENDED USE

Elaboration of the calibration curve for the quantitative determination of Alpha-1 Microglobulin (A1m), in human urine, by immunochemical methods.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, bound to polystyrene particles, when combined with the antigens (Ag) of the patient sample, form insoluble compounds causing a change in the absorbance and dispersion of the light, proportional to the antigen concentration, which can be quantified by turbidimetric (TIA) or nephelometric (NIA) method, by comparison with calibrators of known concentration.

CONTENTS - COMPOSITION - PREPARATION

- Calibrator Level 1: CAL 1 U-A1m
  REF TD-42832-1 CONT 1 ml
- Calibrator Level 2: CAL 2 U-A1m
  REF TD-42832-2 CONT 1 ml
- Calibrator Level 3: CAL 3 U-A1m
  REF TD-42832-3 CONT 1 ml
- Calibrator Level 4: CAL 4 U-A1m
  REF TD-42832-4 CONT 1 ml
- Calibrator Level 5: CAL 5 U-A1m
  REF TD-42832-5 CONT 1 ml
- Calibrator Level 6: CAL 6 U-A1m
  REF TD-42832-6 CONT 1 ml

The calibrators are human A1m solutions.
As preservatives, the calibrators contain <0.1% (1 g/l) Sodium Azide (NaN_3), <0.02% (0.2 g/l) Methylisothiazolone and <0.02% (0.2 g/l) Bromonitrodioxane.
The calibrators are ready for use and require no preparation.
Before each use it is convenient that the calibrators are homogenized, shaking them gently avoiding the formation of foam or bubbles.
It is always advisable to bring the calibrators to room temperature before use.
The values of the calibrators are lot dependent and are indicated in the table of values of their Instructions for Use.

WARNINGS - PRECAUTIONS

- Sodium Azide is toxic. Even if at the concentrations present neither Sodium Azide nor the other preservatives are harmful, take the necessary precautions to avoid accidental ingestion or contact with the eyes.
- Sodium Azide can react with lead or copper to give explosive compounds. For disposal it is recommended to rinse with plenty of running water to avoid accumulation in drains.
- Materials of human origin have been tested and found negative for the presence of HBsAg, HCV, and anti-HIV 1 and 2 antibodies.
- Since the absence of infectious agents can not be proven with absolute certainty, components containing materials of human or animal origin must be handled with caution, as potentially infectious, following the recommended safety standards for biological risk.
- Do not mix components belonging to different lot kits.
- Clinical diagnosis should not be based on the results of a single test, but should always integrate all relevant clinical and laboratory data.

STORAGE - SHELF LIFE

- Store refrigerated at +2...+8ºC. Do not freeze, as the functionality of the calibrators may be altered.
- Properly stored and unopened, the calibrators are stable until the expiration date indicated on the label.
- Once opened, the shelf life of the calibrators is at least 4 weeks, provided that after each use they are stored immediately in the original containers, tightly capped and refrigerated at +2...+8ºC. This information should be taken as a guideline given that, obviously, the shelf life depends on the particular environmental and use conditions, which may differ from those of the stability studies carried out.

MATERIALS NEEDED, NOT SUPPLIED

The calibrators are intended to be used in conjunction with the Reagents and Controls:

- 3diag - U-A1m - TIA — REF TD-42831
- 3diag - U-A1m - CONTROL — REF TD-42833

TRACEABILITY

Given that certified reference materials are not available, values are referred to internal standards based on highly purified proteins.

SYMBOLS

In addition to the harmonized symbols provided on the European Standard EN 980:2008, in the labels and instructions of use has been used the complementary symbology proposed(1) by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

(1) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

CAL	Calibrator
n	Level n (n=1..6)
U-A1m	Alpha-1 Microglobulin - Urine
CONT	Contents

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Reagents for professional use,
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3diag - U-A1m - CONTROL

Alpha-1 Microglobulin - Urine
Controls (2 lev.)
REF **TD-42833**

INTENDED USE

Internal controls, with assigned values, for the quantitative determination of Alpha-1 Microglobulin (A1m), in human urine, by immunochemical methods.

PRINCIPLE OF THE METHOD

The specific antibodies (Ab) of the reagent, bound to polystyrene particles, when combined with the antigens (Ag) of the patient sample, form insoluble compounds causing a change in the absorbance and dispersion of the light, proportional to the antigen concentration, which can be quantified by turbidimetric (TIA) or nephelometric (NIA) method, by comparison with calibrators of known concentration.

CONTENTS - COMPOSITION - PREPARATION

- High Control: CONTROL | H | U-A1m
  REF TD-42833-H — CONT 1 ml
- Low Control: CONTROL | L | U-A1m
  REF TD-42833-L — CONT 1 ml

The controls are ready for use and require no preparation.
Before each use it is convenient that the controls are homogenized, shaking them gently avoiding the formation of foam or bubbles.
The controls are human A1m solutions.
As preservatives, the controls contain <0.1% (1 g/l) Sodium Azide (NaN_3), <0.02% (0.2 g/l) Methylisothiazolone and <0.02% (0.2 g/l) Bromonitrodioxane.
The values of the controls are lot dependent and are indicated in the table of values of their Instructions for Use.

WARNINGS - PRECAUTIONS

- Sodium Azide is toxic. Even if at the concentrations present neither Sodium Azide nor the other preservatives are harmful, take the necessary precautions to avoid accidental ingestion or contact with the eyes.
- Sodium Azide can react with lead or copper to give explosive compounds. For disposal it is recommended to rinse with plenty of running water to avoid accumulation in drains.
- Materials of human origin have been tested and found negative for the presence of HBsAg, HCV, and anti-HIV 1 and 2 antibodies.
- Since the absence of infectious agents can not be proven with absolute certainty, components containing materials of human or animal origin must be handled with caution, as potentially infectious, following the recommended safety standards for biological risk.
- Do not mix components belonging to different lot kits.
- Clinical diagnosis should not be based on the results of a single test, but should always integrate all relevant clinical and laboratory data.

STORAGE - SHELF LIFE

- Store refrigerated at +2...+8ºC. Do not freeze, as the functionality of the controls may be altered.
- Properly stored and unopened, the controls are stable until the expiration date indicated on the label.
- Once opened, the shelf life of the controls is at least 4 weeks, provided that after each use they are stored immediately in the original containers, tightly capped and refrigerated at +2...+8ºC. This information should be taken as a guideline given that, obviously, the shelf life depends on the particular environmental and use conditions, which may differ from those of the stability studies carried out.

MATERIALS NEEDED, NOT SUPPLIED

The controls are intended to be used in conjunction with the Reagents and Calibrators:

- 3diag - U-A1m - TIA — REF TD-42831
- 3diag - U-A1m - CAL SET — REF TD-42832

PROCEDURE

Follow the Instructions for Use of the analyzer used to program and calibrate an assay, with the general parameters recommended in the Instructions for Use of the Reagents.

TRACEABILITY

Given that certified reference materials are not available, values are referred to internal standards based on highly purified proteins. Traceability is ensured by measuring the A1m in the *European Reference Material ERM-DA470k/IFCC* (*Institute for Reference Materials and Measurements, IRMM*).

SYMBOLS

In addition to the harmonized symbols provided on the European Standard EN 980:2008, in the labels and instructions of use has been used the complementary symbology proposed[1] by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

(1) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

CONTROL	Control
H	High
L	Low
U-A1m	Alpha-1 Microglobulin - Urine
CONT	Contents

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13th July 2019.
Modifications highlighted in blue.