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 - RBP - TIA

Retinol Binding Protein
for Turbidimetry
REF TD-42671

INTENDED USE

Quantitative determination of Retinol Binding Protein (RBP), in human serum and 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 | RBP
  REF TD-42671-RA ▽ 100 test (*1) - 3.5 ml
  Anti-RBP antibodies, bound to polystyrene particles.
- Reaction Buffer: BUF | RBP
  REF TD-42671-BF ▽ 100 test (*1) - 20 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 540...600 nm, and accessories: reagent containers, cuvettes, etc..
- 3diag - S-RBP - CAL SET — REF TD-42662-S
- 3diag - RBP - CAL — REF TD-42672
- 3diag - RBP - CONTROL — REF TD-42663
- 3diag - S-RBP - CONTROL — REF TD-42673-S

SAMPLES

- Fresh Serum.
  Samples with presence of fibrin should be centrifuged.
  Do not use hemolyzed, lipemic or contaminated samples.
- Fresh Urine.
  Samples should be centrifuged.

In bibliography(1) it is reported the following stability :

- Serum: Refrigerated: 7 days - Frozen: 90 days
- Urine: Refrigerated/Frozen: 7 days

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 - Serum/Urine

- ①Dispense and mix:
  - Serum: Sample/Control: 15 ul (diluted 1:30)
    Calibrator: 15 ul (see dilution factor in Calibration Parameters)
  - Urine: Sample/Control: 15 ul (diluted 1:5)
    Calibrator: 15 ul (see dilution factor in Calibration Parameters)
  - BUF | RBP 180 ul
- ②Incubate a fixed time between 1 and 5 minutes
- ③Dispense and mix:
  - REAG | Ab | RBP 30 ul
- ④Read absorbance A1 (Blank) at 540...600 nm
- ⑤Incubate a fixed time of about 5 minutes
- ⑥Read absorbance A2 (Final Point) at 540...600 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 saline, or by programming a larger sample dilution in the analyzer, to recover a value close to the midpoint of the measurement range

Calibration Parameters - Serum/Urine

- Serum:
  If using the 3diag - S-RBP - CAL SET program a dilution factor of 1:5.
  If using the 3diag - RBP - CAL program the following dilutions: 1:5, 1:7, 1:15, 1:30, 1:60 and 1:120.
- Urine:
  If using the 3diag - S-RBP - CAL SET program a dilution factor of 1:5.
  If using the 3diag - RBP - CAL program the following dilutions: 1:5, 1:10, 1:20, 1:40, 1:120 and 1:700 (alternatively, saline can be used as the lowest calibration point - zero value).
- It is recommended to use saline as diluent.

- 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 assays is given in the Technical Reports, 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 the **3diag - RBP - CONTROL** or the **3diag - S-RBP - 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.

Values are also referred to commercial calibrators *N Protein Standard SL* (REF: *OQIM13*, LOT: *083643*) from *Siemens Healthcare*.

Traceability is ensured by measuring the RBP in the *European Reference Material ERM-DA470k/IFCC* (*Institute for Reference Materials and Measurements*, *IRMM*).

REFERENCE INTERVALS

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

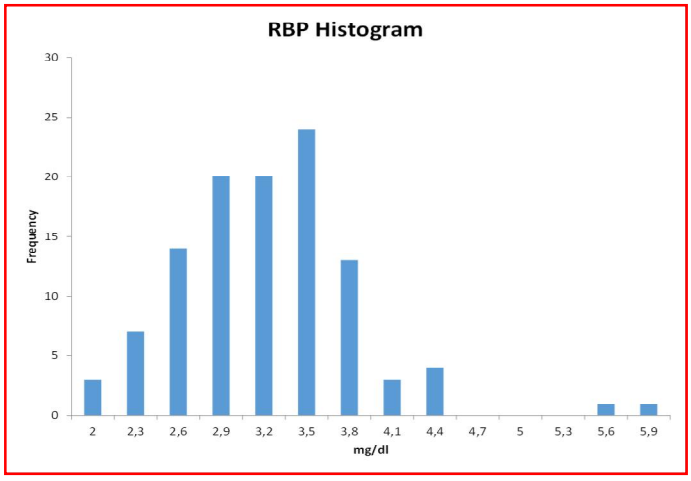
Serum

The bibliography reports variable reference values between publications, depending on the method and standardization used and the population analyzed:

- between 1.5 to 6.7 mg / dl [(1)],
- 2.65 to 6.0 mg/dl for men and 1.9 to 4.61 mg/dl for women[(2)],
- 3.0 to 6.0 mg/dl[(3)(4)(5)],
- and 2.0 to 5.0 mg/dl[(6)].

Analyzing serum samples from 110 presumably healthy adult patients from the Barcelona area, without stratifying them by age or sex, the following results have been obtained (see table and histogram - values based on *Trimero Diagnostics*' internal standardization):

units	mean	SD	range	95 percentile	90 percentile
mg/dl	3.085	0.624	1.91 - 5.63	2.08 - 4.25	2.15 - 4.18



Urine

The bibliography[(1)] reports that an excretion greater than 0.163 mg/24h or 0.130 mg/g-creatinine can be considered significant.

CLINICAL SIGNIFICANCE

Serum RBP is elevated in Diabetes Mellitus tipo 2, obesity, metabolic syndrome, cardiovascular disease and chronic kidney disease. Decreased values due to proteinuria, hepatocellular disease, hyperparathyroidism, cystic fibrosis or other causes, can pose a serious threat to transportation of Vitamin A to tissues.

Urinary RBP results a good marker of tubular dysfunction, due to any cause or pathology, given its good sensitivity, stability and high dynamic range (complete failure of tubular reabsortion increases its excretion in a factor of 10^4-10^5 times the normal values).

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[(7)] by the *EDMA* (*European Diagnostic Manufacturers Association*), whose meaning is detailed below.

REAG	Reagent
Ab	Antibody / Antiserum
BUF	Buffer
RBP	Retinol Binding Protein

BIBLIOGRAPHY

(1) *Mayo Medical Laboratories* website (www.mayomedicallaboratories.com), date of consultation: 18th April 2017.
(2) Miura N. et al.: "*Evaluation of the reference range of retinol-binding protein (RBP) levels by the latex turbidimetric immunoassay*", Rinsho Byori 2009; 57(3): 195-9.
(3) Siemens Heathcare Diagnostics Products GmbH: "*Sistema BN° II - Protocolos de Ensayo - Versión 2.4*", 2009/03.
(4) *ARUP Laboratories* website (www.aruplab.com), date of consultation: 24th January 2019.
(5) Forga L. et al.: "*Low Serum Levels of Prealbumin, Retinol Binding Protein, and Retinol Are Frequent in Adult Type 1 Diabetic Patients*", Journal of Diabetes Research 2016; Article ID 2532108.
(6) Bernard AM. et al.: "Assessment of Urinary Retinol-Binding Protein as an Index of Proximal Tubular Injury", Clin. Chem. 33/6, 775-779 (1987).
(7) EDMA Labelling Task Force: "*EDMA Symbols for IVD Reagents and Components - Revision, October 2009*".

TEXT REVISION DATE

25th July 2022.

Modifications highlighted in blue.

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CE IVD

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INSTRUCTIONS FOR USE

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

3diag - RBP - CAL

Retinol Binding Protein
Calibrator
REF TD-42672

INTENDED USE

Elaboration of the calibration curve for the quantitative determination of Retinol Binding Protein (RBP), in human serum and 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 : CAL | RBP
  REF TD-42672 CONT 1 ml

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.
The calibrators are human serum solutions, delipidated, filtered by 0.2 µm.
As preservative, the calibrators contain <0.1% (1 g/l) Sodium Azide (NaN_3).
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 concentration present Sodium Azide is not 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 - RBP - TIA — REF TD-42671
- 3diag - RBP - CONTROL — REF TD-42663

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 RBP 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*".

CAL	Calibrator
RBP	Retinol Binding Protein
CONT	Contents

TEXT REVISION DATE

7th May 2019.
Modifications highlighted in blue.

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INSTRUCTIONS FOR USE

Reagents for professional use,
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3diag - RBP - CONTROL

Retinol Binding Protein
Controls (serum+urine) (2+2 lev.)
TD-42663
(Product included in REF TD-42660)

INTENDED USE

Internal controls, with assigned values, for the quantitative determination of Retinol Binding Protein (RBP), in human serum and 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 - serum: CONTROL | H | Sr-RBP
  REF TD-42663-SH — CONT 1 ml
- Low Control - serum: CONTROL | L | Sr-RBP
  REF TD-42663-SL — CONT 1 ml
- High Control - urine: CONTROL | H | Ur-RBP
  REF TD-42663-UH — CONT 1 ml
- Low Control - urine: CONTROL | L | Ur-RBP
  REF TD-42663-UL — CONT 1 ml

The controls are human serum solutions, delipidated, filtered by 0.2 µm.
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 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.
It is always advisable to bring the controls to room temperature before use.
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 - RBP - 800 — REF TD-42661
- 3diag - RBP - TIA — REF TD-42671
- 3diag - RBP - CAL SET — REF TD-42662
- 3diag - RBP - CAL — REF TD-42672

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.
For some analyzers, to process the controls it may be necessary to deactivate the analyzer's clot detection system.

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 RBP 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
Sr-RBP	Retinol Binding Protein - serum
Ur-RBP	Retinol Binding Protein - urine
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TEXT REVISION DATE

28th July 2020.