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Instructions for use

5-HIAA ELISA

REF **BA E-1900**

2°C 8°C Σ 96 IVD CE

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1. Introduction

1.1 Intended use and principle of the test

Enzyme immunoassay for the quantitative determination of 5-Hydroxyindolacetic acid (5-HIAA) in urine. The determination of 5-HIAA helps in the diagnosis of carcinoids.
The quantitative determination of 5-HIAA follows the basic principles of a competitive enzyme immunoassay.
First, 5-HIAA is chemically derivatized by a methylation step. The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The methylated analyte in the standards, controls and samples compete with the solid phase bound analyte for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user. This in-vitro diagnostic is for professional use only.

1.2 Clinical application

5-Hydroxyindolacetic acid (5-HIAA) is a metabolite of the serotonin pathway [1, 2]. Serotonin and its major urinary metabolite 5-HIAA, is produced in excess by most enterochromaffin cells from carcinoid tumors, especially those associated with the carcinoid syndrome. Several studies and publications show that analysis of urinary 5-HIAA is important in diagnosis of carcinoid patients [1-8].
Therapeutic consequences should never be based on laboratory results alone, even if these results are assessed in accordance with the quality criteria of the method. Any laboratory result is only a part of the total clinical picture of the patient.
Only in cases where the laboratory results are in an acceptable agreement with the overall clinical picture of the patient, it can be used for therapeutic consequences.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for a certain type of sample as indicated in Intended Use (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) must be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) If serious incidents should occur in connection with this product, they should be reported to the manufacturer and the competent national authorities.
(6) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
(7) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
(8) Standards, Controls and specimen samples should be assayed in duplicate.
(9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
(10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(15) For information about hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(16) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
(17) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(18) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided

that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

(19) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but must be correlated to other diagnostic tests and clinical observations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances and proper handling of specimens

Please note the sample collection! It cannot be excluded that high acid concentrations lead to incorrect results.

2.2.2 Drug and food interferences

Foods generally rich in serotonin such as bananas, pineapple, plums, kiwi fruit, tomatoes, avocados, various nuts, and chocolate should be avoided a few days before sample collection.
Drugs/substances such as imipramine, isoniazid, isocarboxazid, methyldopa, levodopa, MAO-inhibitors, general OTC-medication, alcohol, paracetamol, diazepam, oxprenolol, atenolol, phenothiazines, indomethacin, naproxen, reserpine, glyceryl-guaiacolate have an influence on urinary 5-HIAA levels and should be discontinued a few days before.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents of the kit

BA D-0024	**REAC-PLATE**	**Reaction Plate** – ready to use
Content:	1 x 96 well plate, empty, in a resealable pouch	
BA D-0090	**FOILS**	**Adhesive Foil** – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	1 x 4 foils	
BA E-0030	**WASH-CONC** **50x**	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
Hazardous ingredients:	2-methyl-2H-isothiazol-3-one	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P280 Wear protective gloves. P302+P352 IF ON SKIN: Wash with plenty of water. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	

BA E-0041	**DILUENT**	**Diluent** – ready to use
Content:	Acidic buffer with non-mercury preservative	
Volume:	1 x 22 ml/vial, white cap	
BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	
BA E-0931	**IU SER 5-HIAA**	**Serotonin 5-HIAA Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable pouch with desiccant	
BA E-1910	**5-HIAA-AS**	**5-HIAA Antiserum** – ready to use
Content:	Rabbit anti-5-HIAA antibody, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species is rabbit	
BA E-1913	**ASSAY-BUFF**	**Assay Buffer** – ready to use
Content:	TRIS containing buffer with non-mercury preservative	
Volume:	2 x 55 ml/vial, green cap	
BA E-1937	**METHYL-BUFF**	**Methylation Buffer** – ready to use
Content:	Methanol and dimethyl sulfoxide	
Volume:	1 x 11 ml/vial, brown cap	
Hazard pictograms:	 GHS02 GHS06 GHS08	
Signal word:	Danger	
Hazardous ingredients:	Methanol	
Hazard statements:	H301+H311+H331 Toxic if swallowed, in contact with skin or if inhaled. H370 Causes damage to organs (eye, central nervous system).	
Precautionary statements:	P260 Do not breathe fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection. P308+P311 IF exposed or concerned: Call a POISON CENTER or doctor. P403+P233 Store in a well-ventilated place. Keep container tightly closed. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-1939	**METHYL-REAG**	**Methylation Reagent** – ready to use
Content:	Methylation reagent in hexane	
Volume:	1 x 5 ml/vial, red cap	
Hazard pictograms:	 GHS02 GHS06 GHS08 GHS09	
Signal word:	Danger	
Hazardous ingredients:	Hexane, branched and linear, (Trimethylsilyl)diazomethane	

Hazard statements:	H304 May be fatal if swallowed and enters airways. H330 Fatal if inhaled. H350 May cause cancer. H361f Suspected of damaging fertility. H370 Causes damage to organs (lungs, inhalation).
Precautionary statements:	P201 Obtain special instructions before use. P260 Do not breathe mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P304+P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P310 Immediately call a POISON CENTER or doctor. P331 Do NOT induce vomiting.

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/ Cap	Concentration [mg/l] 5-HIAA	Concentration [mmol/l] 5-HIAA	Volume/ Vial
BA E-1901	**STANDARD A**	white	0	0	4 ml
BA E-1902	**STANDARD B**	yellow	0.5	2.63	4 ml
BA E-1903	**STANDARD C**	orange	1.5	7.88	4 ml
BA E-1904	**STANDARD D**	blue	5	26.3	4 ml
BA E-1905	**STANDARD E**	grey	15	78.8	4 ml
BA E-1906	**STANDARD F**	black	50	262.5	4 ml
BA E-1951	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-1952	**CONTROL 2**	red			4 ml
Conversion:	5-HIAA [mg/l] x 5.25 = 5-HIAA [µmol/l]				
Content:	Acidic buffer spiked with defined quantity of 5-HIAA				

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)
- Reaction tubes, at least 3 ml, Polypropylene/Polystyrol

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 20 – 300 µl; 1 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 – 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Vortex mixer
- Ventilated hood

5. Sample collection, handling and storage

24-hour urine

24-hour urine sample is used for analysis. Over a defined period of 24 hours, all urine is collected in a bottle with acid (10 – 15 ml 6 M hydrochloric acid) provided for stabilization. During the collection period, the collected sample must always be stored in a cool place protected from light (2 – 8 °C). A creatinine determination for normalization is required.

Storage for a short period up to 7 days is at 2 – 8 °C. Storage for a longer period up to 6 months is at -20 °C. Repeated freezing and thawing should be avoided. Avoid direct sunlight!

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.

If the product is prepared in parts, unused wells in Reaction Plates should be covered to avoid contamination. After preparation, the used wells must be labelled to prevent double use.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

⚠ *The Methylation Reagent is volatile. If possible, please pipette the Methylation Reagent with a repetitive pipette and make sure that the vial is recapped immediately after pipetting.*

6.1 Preparation of reagents and further notes

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50x** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

Serotonin 5-HIAA Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Predilution of standards, controls and samples

1.	Pipette **50 µl** of **standards, controls** and **urine samples** into the respective wells of the **REAC-PLATE**.
2.	Pipette **200 µl** of the **DILUENT** into all wells.
3.	Shake for **1 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). **20 µl** are needed for the **methylation.**

6.3 Methylation

1.	Pipette **20 µl** of the **prediluted standards**, **controls** and **urine samples** into the respective **reaction tubes.**
⚠	*The following steps 2 – 5 must be performed in a ventilated hood!*
2.	Pipette **100 µl** of **METHYL-BUFF** into all reaction tubes.
3.	Add **20 µl** of **METHYL-REAG** to each reaction tube and **mix each reaction tube immediately after addition of the Methylation Reagent**.
4.	Cover all reaction tubes and **methylate** for **20 min** at **RT** (20 – 25 °C).
5.	Pipette **1000 µl** of **ASSAY-BUFF** into all reaction tubes. *After this step the use of a ventilated hood is not necessary anymore!*
⚠	**Proceed** with the **ELISA** (Chapter 6.4) **immediately** as the methylated standards, controls and samples are only stable for 1 hour!

6.4 5-HIAA ELISA

1.	Pipette **25 µl** of the **methylated standards, controls** and **samples** into the appropriate wells of the **LU** **SER** **5-HIAA** microtiter strips.
2.	Pipette **50 µl** of the **5-HIAA-AS** into all wells.
3.	Cover plate with **FOILS** and incubate for **1 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
4.	Remove the foil. Discard or aspirate the content of the wells. Wash the plate **4 times** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **CONJUGATE** into all wells.
6.	Cover plate with **FOILS** and incubate for **1 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
7.	Remove the foil. Discard or aspirate the content of the wells. Wash the plate **4 times** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Pipette **100 µl** of the **SUBSTRATE** into all wells and incubate for **20 – 30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
⚠	***Avoid exposure to direct sunlight!***
9.	Add **100 µl** of the **STOP-SOLN** to all wells and shake the microtiter plate shortly.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	5-HIAA
	0.4 – 50 mg/l

The standard curve, which can be used to determine the concentration of the unknown samples, is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis) using a concentration of 0.001 mg/l for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

Urine samples and controls

The concentrations of the samples and controls can be read directly from the standard curve.
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with water (deionized, distilled, or ultra-pure) and must be re-assayed. For the calculation of the concentrations this dilution factor has to be taken into account.
The amount of 5-HIAA normalized to creatinine is calculated as following:
mmol/mol creatinine = [5-HIAA (mg/l) x 191 (mg/mmol)] / [creatinine (mg/dl) x 113 (mg/mmol) x 100].

Conversion:

5-HIAA [mg/l] x 5.25 = 5-HIAA [µmol/l]

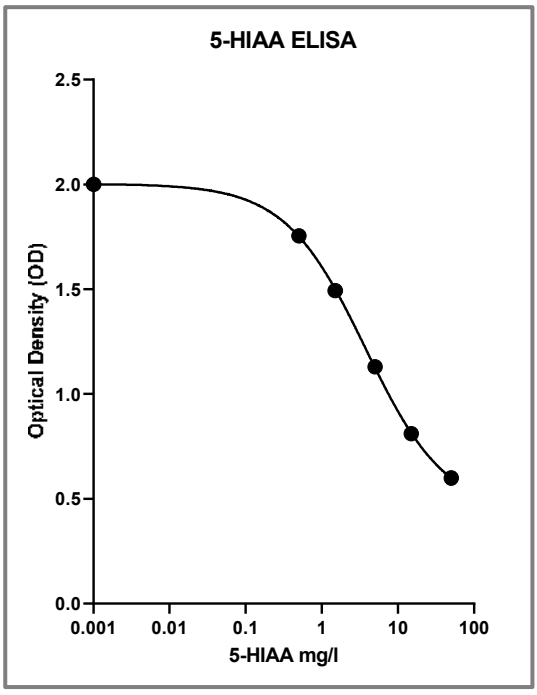
7.1 Expected reference value

It is strongly recommended that each laboratory should determine its own reference values.
The expected reference value was determined in a study by Meijer et al 2000 [2]. Normalization is performed on creatinine, which is an indication of urinary dilution.

	5-HIAA in urine
Reference value (ULN)	2.8 mmol/mol creatinine
Typical pathological range	up to 380 mg/24h

7.2 Typical standard curve

⚠ *Example: Do not use for calculation!*



8. Control samples

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. Commercially obtained control samples should be treated like unknown samples. Control samples should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity	**5-HIAA**
Limit of Blank (LOB)	0.16 mg/l
Limit of Detection (LOD)	0.23 mg/l
Limit of Quantification (LOQ)	0.40 mg/l

Analytical Specificity (Cross Reactivity)	
Substance	**Cross Reactivity [%]**
	5-HIAA
Serotonine	7.6
5-Hydroxy-DL-Tryptophane	2.3
Tryptamine	< 0.1
Melatonine	< 0.1
5-Methoxytryptamine	< 0.1
DL-Vanillic mandelic acid	< 0.1
Homovanillic acid	< 0.1

Precision					
Intra-Assay			**Inter-Assay**		
n = 24			n = 9		
Sample	Mean ± SD [mg/l]	CV [%]	Sample	Mean ± SD [mg/l]	CV [%]
1	1.1 ± 0.15	13.3	1	11.3 ± 1.3	11.9
2	1.9 ± 0.18	9.3	2	4.8 ± 0.6	12.8
3	5.3 ± 0.48	9.0	3	3.1 ± 0.3	8.6
4	14.3 ± 1.2	8.7	4	7.3 ± 0.8	10.8
			5	19.0 ± 2.2	11.4

Lot-to-Lot					
	Sample	Reference Range [mg/l]	mean ± SD [mg/l]	mean ± SD Recovery [%]	CV [%]
5-HIAA in artificial matrix (n = 3)	1	3.0 – 7.0	4.9 ± 0.36	98.0 ± 7.2	7.4
	2	9.0 – 21.0	14.6 ± 1.3	97.3 ± 9.0	9.2

Recovery			
	Range [mg/l]	Range [%]	Mean [%]
Urine	0.8 – 40.5	86 – 93	90

Linearity			
	Serial dilution up to	Range [%]	Mean [%]
Urine	1:10	98 – 112	105

Method Comparison: ELISA vs. XLC-MS/MS [1]	
Urine	ELISA = 0.9749 * (XLC-MS/MS) – 0,0868; r^2 = 0,98; n = 95

Diagnostic Performance [2]			
Diagnostic Specificity [%]	Diagnostic Sensitivity [%]	Positive Predictive Value (PPV) [%]	Negative Predictive Value (NPV) [%]
89	68	58	93
Positive Likelihood Ratio (LR+)		Negative Likelihood Ratio (LR-)	
6.2		0.36	

9.2 Metrological Traceability

The values assigned to the standards and controls of the 5-HIAA ELISA are traceable to SI Units by weighing with quality-controlled analyte.

Standards and Controls	Uncertainty [%]
	2.4

5-HIAA ELISA	Concentration [mg/l]	Expanded Uncertainty [%] k = 2*
	11.3	24.3
	4.8	26.0
	3.1	17.8
	7.3	22.1
	19.0	23.3

* This defines an interval about the measured result that will include the true value with a probability of 95%.

10. References/Literature

1. de Jong, W.H., et al., *Urinary 5-HIAA measurement using automated on-line solid-phase extraction-high-performance liquid chromatography-tandem mass spectrometry.* J Chromatogr B Analyt Technol Biomed Life Sci, 2008. **868**(1-2): p. 28-33.
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3. Formica, V., et al., *The prognostic role of WHO classification, urinary 5-hydroxyindoleacetic acid and liver function tests in metastatic neuroendocrine carcinomas of the gastroenteropancreatic tract.* Br J Cancer, 2007. **96**(8): p. 1178-82.
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6. Padelli, M., et al., *[Determination of thresholds values for platelet serotonin and urinary 5-HIAA concentrations for the biological diagnosis of digestive neuroendocrine tumors].* Ann Biol Clin (Paris), 2019. **77**(2): p. 161-168.
7. Tirosh, A., et al., *Prognostic Utility of 24-Hour Urinary 5-HIAA Doubling Time in Patients With Neuroendocrine Tumors.* Endocr Pract, 2018. **24**(8): p. 710-717.
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For updated literature or any other information please contact your local supplier.
The summary of safety and performance according to article 29 of regulation (EU) 2017/746 can be downloaded from the website www.ldn.de.

11. Changes

Version	Release Date	Chapter	Change
17.0	2022-05-17	All 1. 2.1 2.2.2 5. 7. 9.1 9.2 10. 11.	- The IFU was revised according to the IVDR regulation (EU) 2017/746 - Introduction - Procedural notes, guidelines and warnings - Drug and food interferences - Sample collection and storage - Measuring range, expected reference value and typical standard curve have been updated - Performance data updated and Lot-to-Lot added - Metrological traceability added - References/Literature updated - Changes added
18.0	2023-09-13	4.1 4.1	- Hazard labelling updated according to SDS - BA E-1939 Methylation Reagent now with black cap
19.0	2023-11-14	4.1	- BA E-1939 Volume and cap colour changed
20.0	2025-06-02	2.1 4.1 5 7 9.2	- Updated - BA E-0040: Hazard labelling updated according to SDS - Note added regarding creatinine determination - Note added to the dilution factor in the calculation and to creatinine - Metrological Traceability updated

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE marking of conformity
	Caution		Catalogue number		Distributor
	Date of manufacture		Do not re-use		