M-ALBU-CHECK-1

Quantitative determination of microalbumin in urine samples

- FOR EASY READER® AND EASY READER+® USE ONLY -

- PATENTED TEST –

Ref.: 27091-3L (20 tests) & Ref.: 27091-3L-10T (10 tests)

I-INTENDED PURPOSE

The M-ALBU-CHECK-1 is a rapid screening test for the determination of microalbumin level in human urine samples to be used as a tool by medical healthcare professionals in assessing renal function. The sole measurement of microalbumin concentration is not sufficient to diagnose kidney deficiency or renal problem due to diabetes or hypertension.

II- PRINCIPLE

In patients with diabetes mellitus, a slight albuminuria (microalbuminuria) is predictive of diabetic nephropathy (1, 2), early overall mortality (3) and increased cardiovascular mortality (4, 5).
In non-diabetic populations, microalbuminuria has implicated association with hypertension (6), obesity (7), blood glucose concentrations (8) and plasma triglyceride concentration.
M-ALBU-CHECK-1 is a rapid quantitative test for the detection of albumin in urine samples. The method relies on competitive binding of anti-albumin monoclonal antibodies gold conjugate with free albumin present in the urine sample and membrane coated albumin.
Depending on microalbumin concentration in the sample, different lines will appear on the test, allowing the quantitative measurement of microalbumin, when used in combination with the VEDALAB rapid test reader.

III- M-ALBU-CHECK-1 KIT COMPONENTS

Each kit contains everything needed to perform 10 or 20 tests.

1- M-ALBU-CHECK-1 reaction devices:	10	20
2- Disposable plastic pipettes:	10	20
3- Plastic tubes containing 2 mL of buffer:	10	20
4- Diluent dropper bottle:	5 mL	10 mL
5- Instructions leaflet:	1	1

6- Controls (Optional):

Positive control (ref. V2900, freeze-dried) and Negative control (ref. V2901, liquid): a freeze-dried preparation of a non-infectious compound in diluted human serum, tested and found negative for anti-HIV, anti-HCV and HBs antigen, containing 0.05 % sodium azide is optionally available as a positive and negative control (1x 0.5 mL). The concentration range is indicated on the vial label.

Material required but not provided

- Automatic precision pipette for sampling (**10 µL**).
- Plastic tubes (Eppendorf® tubes or similar).
- Timer.

IV- STORAGE AND STABILITY

1- All M-ALBU-CHECK-1 kit components should be stored at room temperature (+4°C to +30°C) in the sealed pouch.
2- ***Do not freeze the test kit***.
3- The M-ALBU-CHECK-1 kit is stable until the expiry date stated on the package label.

V- PRECAUTIONS

1- This test is designed for *in vitro* diagnostic use and professional use only.
2- Read carefully the instructions before using this test.
3- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1% solution of sodium hypochlorite for one hour before disposal.
4- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
5- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
6- Avoid any contact between hands and eyes or nose during specimen collection and testing.
7- Do not use beyond the expiry date which appears on the package label.
8- Do not use a test from a damaged protective wrapper.

VI- SPECIMEN COLLECTION AND PREPARATION

1- M-ALBU-CHECK-1 test should be performed on urine sample.
2- The urine specimen should be collected under the standard laboratory conditions in a clean container free of detergent.
3- For optimal detection, a first morning urine specimen is preferred, since this sample type is reportedly less biologically variable than random samples (9).
4- If testing is not immediate, the specimen should be refrigerated (+2°C to +8°C) for up to 24 hours. In such case, bring the specimen to room temperature prior to testing.
If testing is delayed more than 24 hours, the specimen should be frozen. The frozen specimen must be completely thawed, thoroughly mixed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.

VII- ASSAY PROCEDURE

<u>IMPORTANT</u>: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

A) Control preparation (<u>no dilution required</u>)

- <u>Positive control:</u> Wait for 15 minutes after freeze-dried dissolving and proceed as indicated in c) "Controls and samples testing".
- <u>Negative control:</u> Ready to use. Proceed as indicated in C) "Controls and samples testing".
- The expected concentration level (**in µg/mL**) is indicated on the vial label and obtained result must match the indicated value. The concentration level can change slightly depending on lot number.

- <u>Positive control:</u> The reconstituted vial should be kept between +2°C and +8°C and should be used within 7 days after reconstitution.

- <u>Negative control:</u> After each use, promptly replace the stopper and keep the vial between +4°C to +30°C.

B) Sample preparation (<u>dilution required</u>)

1) Standard dilution

- Label one plastic tube containing 2 mL buffer with patient's name.
- Remove the cap and using a precision pipette add **<u>10 µL</u>** of urine sample into the tube containing the buffer.
- Replace the cap on the tube and mix well for a few seconds.

Please do not discard this diluted sample as a further dilution of the sample might be necessary (see Part VIII A) Linearity).

2) Additional dilution

This step must be performed only in case the microalbumin concentration is over 300 µg/mL.

- Label an empty plastic tube (Eppendorf® tube or similar) with patient's name and "1/15 dilution" statement.
- Add **14** drops (420 µL) of diluent, using the dropper vial into the tube.
- Add one drop (30 µL) of the **already diluted sample** previously prepared for the first microalbumin testing (See Part VII-B 1: Sample preparation).
- Mix well for a few seconds and proceed as indicated in C) "Controls and samples testing".

- The obtained concentration must be multiplied by 15 (x 15) in order to get the right value.

Note: Further dilution could be necessary for samples containing very high levels of albumin. In this case repeat the above steps using the 1/15 diluted sample.

C) Controls and samples testing

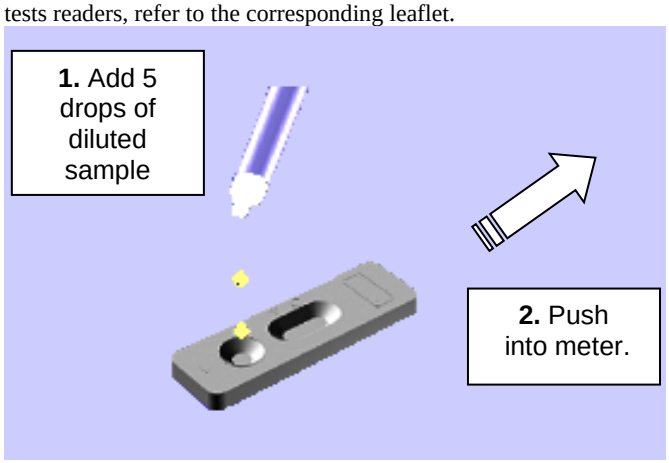
Follow the below instructions or refer to the picture n°1.

1– Allow samples and M-ALBU-CHECK-1 test devices to come to room temperature prior to testing.
2– Remove the reaction device from its protective wrapper by tearing along the split.
3– Label device with the patient's name or control number.

4.1– **Controls:** Dispense 150µL of **undiluted control** (**preparation described in VII. A**) with **lab pipette (disposable tips)** into the sample well of the cassette (▷).
4.2– **Urine sample:** Fill the disposable pipette with **diluted sample** and by holding it vertically, dispense drop-wise into sample well (▷). Add exactly 5 drops (150µL without air bubble) in the sample well (▷).

5– Read the result **(in µg/mL**) after 10 minutes.

For general instructions describing how to use the VEDALAB's rapid tests readers, refer to the corresponding leaflet.



Picture n°1

VIII- PERFORMANCES CHARACTERISTICS

A) Linearity

The measuring range is 2.5 to 5,000 µg/mL and results are given as per table 1.

Microalbumin Concentration (µg/mL)	Reader results (µg/mL)
0 - 2.5	"< 2.5 µg/mL"
2.5 - 300	Quantitative result
300 – 1,000	"> 300 µg/mL"
1,000 – 5,000	"> 1000 µg/mL"
5,000 and over	"> 5000 µg/mL"

Table 1

The linear range being 2.5 to 300 µg/mL, a second measurement with an additional 1/15 dilution of the diluted sample (See Part VII B – 2) Additional dilution) will be necessary, in case an exact microalbumin value is needed for samples over 300 µg/mL

B) Analytical sensitivity

The sensitivity of M-ALBU-CHECK-1 test is close to 2 µg/mL when using the microalbumin ERM-DA470K/IFCC standard. The result will be rendered as "< 2.5 µg/mL".

C) Accuracy

A study has been performed using negative samples spiked with microalbumin standard ref. ERM-DA470K/IFCC. In the range of 2.5 to 300 µg/mL, the obtained optical densities expressed as a function of microalbumin concentrations show the following polynomial curve formula:

$$Y = 20.8 + 2.294\ X - 5.86.10^{-3}\ X^2.$$

The regression coefficient is r = 0.94 demonstrating the good correlation of the microalbumin values obtained using VEDALAB's reader.

D) Comparative Study

A panel of 30 urines samples pre-assayed on ADVIA SIEMENS analyser was tested using the M-ALBU-CHECK-1 rapid test. Results are shown in table 2.

Urines ident.	SIEMENS Advia (µg/mL)	Confidence range		VEDALAB M-ALBU-CHECK-1 (µg/mL)
		Lower limit	Upper limit	
1	**1**	**0.7**	**1.3**	**3.2**
2	**2**	**1.5**	**2.5**	**2.8**
3	2	1.5	2.5	<2.5
4	**2.24**	**1.6**	**2.8**	**4.5**
5	**3**	**2.2**	**3.8**	**5.0**
6	**3**	**2.2**	**3.8**	**5.4**
7	**4**	**3**	**5**	**9.1**
8	**4**	**3**	**5**	**7.5**
9	**4**	**3**	**5**	**8.6**
10	**5**	**3.7**	**6.3**	**8.7**
11	6	4.5	7.5	7.2
12	**6**	**4.5**	**7.5**	**12.9**
13	7	5.2	8.8	7.5
14	**7**	**5.2**	**8.8**	**9**
15	7	5.2	8.8	5.6
16	**7**	**5.2**	**8.8**	**4.3**
17	9	6.7	11.3	10
18	12	9	15	14.4
19	20	15	25	15.2
20	21	15.7	26.3	23.4
21	28	21	35	28.9
22	29	15.7	36.3	32.5
23	37,8	28.3	47.3	40.6
24	53	39.7	66.3	60.4
25	76	57	95	85.3
26	83	62.2	103.8	65.5
27	94	70.5	117.5	96.2

28	110	82.5	137.5	131.7
29	111	83.2	138.8	137
30	266	199.5	332.5	>300

Table 2

A discrepancy is obtained with 12 urines samples (identified by bold typo). Discrepant results are obtained using urine samples containing very low (and therefore not significant) concentration of micro-albumin but nevertheless, both methods indicate the same clinical diagnosis profile (negative).
Therefore, all samples pre-assayed by ADVIA SIEMENS with negative, borderline or pathological micro-albumin concentrations are correctly detected by M-ALBU-CHECK-1.
When considering the diagnostic performance, the sensitivity is 91.7% and the specificity attains 100% with an overall agreement of 96.6%.
In addition, the results show a correlation of 98.4% (CI95%* [96.8 – 99.3]) between the concentration values obtained with VEDALAB and ADVIA SIEMENS methods.

*CI95%: 95% confidence interval

E) Interference study
1- **Glucose:** negative urine spiked with 50 to 250 mmol. of glucose showed repeatedly negative results.

2- **pH:** negative and positive urine were assayed at pH ranging from 2.5 to 9.5.
Obtained results were always in accordance with expected results.

3- **Bilirubin**: negative urine spiked with 25 to 375 µmol. of bilirubin showed repeatedly negative results.

4- **Haemoglobin**: negative urine spiked with 15 to 210 µmol. of haemoglobin showed repeatedly negative results.

5- **Immunoglobulins**: negative urine spiked with 30 to 200 µg/mL of gammaglobulins showed repeatedly negative results.

F) Hook effect
Negative urine spiked with 100 to 6,000 µg/mL of human albumin showed repeatedly positive results: Reader rendered a "> 5000 µg/mL" result. No hook effect was observed.

G) Intra-assay reproducibility
Within run precision was evaluated by using 25 replicates of three commercially available sera containing 4.9, 32.4 and 134 µg/mL of micro-albumin using M-ALBU-CHECK-1 on VEDALAB's reader. The obtained CVs (coefficient of variation) were respectively 13.5%, 8% and 8.7%.

H) Inter-lot reproducibility
Four samples having different concentration levels in albumin were tested in triplicate using three different lots of M-ALBU-CHECK-1 devices. The obtained results are reported in table 3.

	Albumin concentration in µg/mL			
	5	25	100	500
Acceptability range	[3.5-5.8]	[19.1-31.8]	[78.7-131.2]	> 300
Lot 1	3.8	27.8	108.1	> 300
Lot 2	4.8	22.1	98.6	> 300
Lot 3	4.4	22.8	94.4	> 300

Table 3

All results were within the acceptability concentration range for the 3 tested devices lots and the 4 concentration levels samples. The reproducibility performance of M-ALBU-CHECK-1 is 100 %.

IX- LIMITATIONS

1- In case of fever, acute infection, pregnancy or intensive sport practice, the albumin concentration in urine may be increased. Do not run the test in the above conditions.
2- Insufficient (less than 1 litre per day) or excessive liquid absorption during the day before running the test may generate false positive or false negative results.
3- A clean, well-rinsed container should be used for urine collection as detergent may interfere in the normal reaction.
4- This format of test is to be only used with VEDALAB rapid test reader (EASY READER® or EASY READER+®).
5- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.
6- This format of test should not be used for visual reading.
7- As for any diagnostic procedure, the physician should evaluate data obtained by the use of this kit in light of other clinical information.
8- As it is true for any diagnostic method on any measurements through analysers, there is a variability of the obtained results. Therefore, a confidence range of ± 25% should be considered for the final value and for the clinical significance of the result.
9- Do not use the reader for measurements before at least 30 minutes warm-up after having switched it on.

X- BIBLIOGRAPHY

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8- Damsgaard EM, Mogensen CE. Microalbuminuria in elderly hyperglycaemic patients and controls. Diabetic Med 1986: 3 430-5.

9- How JEA, Browning MCK, Fraser CG. Selecting the optimum specimen for assessing slight albuminuria, and a strategy for clinical investigation: novel uses of data on biological variation. Clin Chem 1987; 33: 2034-8.

	Read the instructions before use	IVD	For *in vitro* diagnostic use
+30°C +4°C	Temperature limitations		Do not reuse
	Manufacturer		

Manufactured by VEDALAB - France

CHANGES DESCRIPTION

Changes type:

- N/A	Not Applicable (creation)
- Technical change	Addition, revision and/or removal of information related to the product.
- Administrative	Implementation of non-technical changes noticeable to the end-user.

Changes type	**Change description**
Technical change	Addition: - Chap I intended purpose - Chap VII and IX warm up reader information - Chap VIII D) CI 95%
Administrative change	Addition: 10 tests reference

Note: Minor typographical, grammar, spelling and formatting changes are not reported in the change details.