

# AFP-CHECK-1

## Quantitative determination of alpha-Fetoprotein in whole blood, plasma or serum samples

### FOR EASY READER<sup>®</sup> OR EASY READER+<sup>®</sup> USE ONLY

Ref.: 23091 (20 tests) / Ref.: 23091-10T (10 tests)

#### I- PRINCIPLE

Alpha-fetoprotein (AFP) is a single-chain glycoprotein with a molecular weight of approximately 70,000 (1). It is produced by the fetal yolk sac and proximal structures of the liver and gastrointestinal tract. In the human fetus, AFP is a major serum protein which reaches a level of several milligrams per milliliter at week 12 of gestation and then drops to trace concentration in normal non pregnant adult.

The clinical value of AFP as a tumor marker was not immediately appreciated because the assays used for quantitation were not sensitive enough to detect the nanogram amounts associated with early disease. As more sensitive radioimmune assays became available, the utility of AFP as a tumor marker became increasingly apparent.

Marked increases are found in malignant tumors in childhood, such as hepatoblastomas and nephroblastomas and in hepatocellular carcinoma and certain testicular tumours in adults. Less commonly, malignant tumours of the gastro intestinal tract and other organ systems with massive hepatic metastases are associated with increased concentrations of AFP in serum (2, 3).

The AFP-CHECK-1 test is a rapid quantitative assay for the detection of human AFP in serum, plasma or whole blood. The method employs a unique combination of monoclonal-dye conjugate and polyclonal solid phase antibodies to identify AFP in the test samples with a high degree of specificity.

As the test sample flows through the absorbent device, the labelled antibody-dye conjugate binds to the AFP forming an antibody-antigen complex. This complex binds to the anti-AFP antibody in the positive reaction zone (T) and produces a pink-rose colour band.

In the absence of AFP, there is no line in the positive reaction zone (T). The reaction mixture continues flowing through the absorbent device past the reaction zone (T) and control zone (C). Unbound conjugate binds to the reagents in the control zone (C) producing a pink-rose colour band, demonstrating that the reagents are functioning correctly.

Depending on the AFP concentration level, different lines of different intensities will appear on the reading window allowing the quantitative measurement of AFP when used in combination with the VEDALAB's readers.

#### II- AFP-CHECK-1 KIT COMPONENTS

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

1- AFP- CHECK-1 reaction devices:	10	20
2- Disposable plastic pipettes:	10	20
3- Diluent in a dropper bottle:	2.5mL	5mL
4- Instruction leaflet:	1	1

#### 5- Controls (Optional):

**Positive control ref. V8000 and Negative control ref. V8001:** 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 0.25 mL). The concentration range is indicated on the vial label.

#### III- STORAGE AND STABILITY

1- All AFP-CHECK-1 test components, including optional control before reconstitution, should be stored at any temperature between +4°C and +30°C in their original package.

2- ***Do not freeze the kit.***

3- The AFP-CHECK-1 kit is stable until the expiry date stated on the package label.

#### IV- 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.

#### V- SPECIMEN COLLECTION AND PREPARATION

1- AFP-CHECK-1 test is to be performed on human serum, plasma or whole blood.

2- The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid haemolysis).

**3- If anticoagulant is needed, only citrate, EDTA or heparin should be used.**

4- Each specimen should be treated as if potentially infectious.

**5- Whole blood samples should be tested immediately (< 4 hours). Finger prick samples should be assayed just after collection.**

6- If the test is to be run within 48 hours after collection the specimen should be stored in the refrigerator (+2°C to +8°C). If testing is delayed more than 48 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.

7- In case of cloudiness, high viscosity or presence of particulate matter into the serum specimen, it should be diluted with equal volume (V/V) of diluting buffer (not provided but available upon request) before testing.



## VI- ASSAY PROCEDURE

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

### a) Controls testing

- Wait for 15 minutes after the freeze-dried control has been reconstituted and mix well few seconds (Vortex).
- Add the requested volume (25µL) with **lab pipette (disposable tips)** into the sample well of the cassette and proceed in the same way as for a patient's sample.
- The expected concentration level (**in ng/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.
- **The reconstituted vial should be kept at +2°C to +8°C and should be used within 7 days after reconstitution.**

### b) Samples testing

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

- 1- Allow samples and AFP-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- Fill the serum dropper with specimens (serum or plasma) and by holding it vertically, dispense one drop (25 µL) into sample well. If the whole blood is used, dispense two drops (50 µL) into the sample well (▷) **and wait for the blood sample to be completely absorbed before adding diluent.**
- 5- Hold the diluent vial vertically and slowly add exactly 4 drops of diluent (150 µL) in the sample well (▷) **with an interval of 2-3 seconds between each drop.**
- 6- Read the result (**in ng/mL**) after 10 minutes either using the immediate or countdown reading mode (see corresponding leaflet).

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



Picture n°1

## VII- PERFORMANCES CHARACTERISTICS

### a) Linearity

The measuring range is 10 – 300 ng/mL.

For AFP concentration below 10 ng/mL, the result will be given as “< 10 ng/mL”.

For AFP concentration over 300 ng/mL, the result will be given as “> 300 ng/mL”.

For samples whose concentration is higher than 300 ng/mL, dilute with saline and repeat the assay as per instructions of Part. VI.

### b) Accuracy

A study has been performed using serum samples obtained from dilutions of AFP W.H.O. reference material. Covering a range of 0 to 300 ng/mL. Optical densities expressed as a function of AFP concentrations are described by following linear curve:

$$Y = 33.53 + 1.23 x \quad (r = 0.987)$$

The results show a good correlation ( $r > 0.95$ ) of the values obtained with AFP-CHECK-1 on VEDALAB's readers.

### c) Sensitivity

Concentrations close to 5 ng/mL are detected by AFP-CHECK-1 test. In these cases, results will be rendered as “<10 ng/mL”.

Levels below are generally considered as abnormal values:

- Adult: over 40 ng/mL
- Child (<1 year) : over 30 ng/mL

The test sensitivity was adjusted to 10 ng/mL using commercially available preparations.

**d) Precision**

A panel of 35 human sera samples pre-assayed on the BECKMAN DXI 800 analyser have been tested with AFP-CHECK-1 rapid test. Results were read using the VEDALAB's readers. Results are shown in table I.

Table I

Human sera identification SCIPAC panel (lot : 571-146)	Age	Sex	[AFP] in ng/mL Expected value BECKMAN DXI 800	Confidence range		[AFP] in ng/mL Expected value AFP-CHECK-1
				Lower limit	Upper limit	
5	38	F	1.5	1.125	1.875	<10
4	68	F	2.4	1.8	3	<10
10	68	M	2.8	2.1	3.5	<10
1	69	F	3.5	2.625	4.375	<10
9	55	F	6.1	4.575	7.625	<10
2	50	F	7.3	5.475	9.125	<10
8	67	F	10.6	7.95	13.25	<10
7	47	F	10.7	8.025	13.375	10.96
3	42	F	11.6	8.7	14.5	12.95
6	48	M	14.7	11.025	18.375	14.95
19	53	M	17	12.75	21.25	21.23
15	58	F	18	13.5	22.5	16.94
11	52	F	18.1	13.575	22.625	19.73
14	86	F	18.3	13.725	22.875	21.35
18	65	M	20.4	15.3	25.5	21.33
12	53	F	24.9	18.675	31.125	22.53
20	29	F	28.6	21.45	35.75	30.52
16	51	M	35.3	26.475	44.125	33.01
13	56	F	39.4	29.55	49.25	40.58
17	52	F	40.6	30.45	50.75	31.93
21	58	M	52.8	39.6	66	45.67
25	56	M	53	39.75	66.25	42.75
24	71	F	53.4	40.05	66.75	59.85
22	13	M	54.9	41.175	68.625	59.85
27	5	M	69	51.75	86.25	75
28	81	M	73.1	54.825	91.375	65.53
30	72	M	81.8	61.35	102.25	80.05
29	55	M	84.3	63.225	105.375	68.06
23	73	F	111.2	83.4	139	97.1
26	49	M	156.8	117.6	196	128.36
33	76	M	1334.4	1000.8	1668	>300
32	76	M	1422	1066.5	1777.5	>300
32 diluted 1/5	76	M	284.4	213.3	355.5	246.77
32 diluted 1/6	76	M	237	177.75	296.25	221.99
34	66	M	4084	3063	5105	>300
35	49	M	21395	16046.25	26743.75	>300
<b>31</b>	<b>89</b>	<b>M</b>	<b>443897</b>	<b>332922.75</b>	<b>554871.25</b>	<b>636400</b>

Discrepancy was obtained only with one sample (identified in bold font). Result obtained with rapid test VEDALAB is 43% higher in comparison with the BECKMAN DXI 800.

This sample has a high level of AFP as shown by both methods (443,897 ng/mL). Result with rapid test indicates probably the same clinical diagnosis profile (obvious pathological positive result).

In all of other cases, negative, borderline and pathological samples were clearly detected. Data from the above table show that 99.5 % (CI 95% [99-100])\* of the results (regression coefficient) obtained with the VEDALAB rapid test correlate with the results obtained on the BECKMAN DXI 800 analyser.

\*CI 95%: 95% confidence interval

**e) Hook effect**

No hook effect was observed up to an AFP concentration of 443,897 ng/mL.

**f) Intra-assay reproducibility**

Within run precision was evaluated by using 26 replicates of three commercially available references containing 23.76, 121.67 and 250 ng/mL of AFP as determined with quantitative AFP-CHECK-1 for VEDALAB's reader.

The obtained CVs (coefficient of variation) were respectively equal to 9.30%, 9.48% and 7.45%.

**g) Inter-assay reproducibility**

Between lots reproducibility was determined by using the three control serum samples (Biorad Lyphochek) containing 32.9, 127.8 and 234 ng/mL of AFP respectively measured using three different lots of AFP-CHECK-1 (3 replicates /lot). The obtained CVs (coefficient of variation) are 14.8%, 13.9% and 12.2% respectively.

**VIII- LIMITATIONS**

1- As for any diagnostic procedure, the physician should evaluate data obtained by the use of this test in light of other clinical information.

2- Some serum specimens with high rheumatoid factor (RF) concentration may yield non-specific positive results during testing. Such cases should be identified before testing.

3- The test is designed to eliminate the potential interference of human antibodies to murine IgG (HAMA). However high level of HAMA could give falsely positive results.

4- **Use only fresh whole blood samples (< 4 hours) when test is performed with blood samples. Finger prick samples should be assayed just after collection.**

5- This format of test is to be only used with VEDALAB rapid test readers.

6- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.

7- This format of test should not be used for visual reading.

8- As it is true for any diagnostic method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/- 25% should be considered for the final value and for the clinical significance of the result.

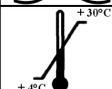
9- Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.

**IX- BIBLIOGRAPHY**

1- **Vessella, R.L., M.A. Santrach, D. Bronson, C.J. Smith, M.J. Klicka, and P.H. Lange.** (1984), "Evaluation of AFP glycosylation heterogeneity in cancer patients with AFP producing tumors". Int. J. Cancer 34 : 309-314.

2- **Lange, P.H. and D. Raghavan.** (1983) "Clinical applications of tumor markers in testicular cancer", p. 111-130. In J.P. Donohue (ed.), Testis tumors. The Williams & Wilkins CO., Baltimore.

3- **Vogelzang, N.J., P.H. Lange, A. Goldman, R.L. Vessella, E.E. Fraley, and B.J. Kennedy.** (1982), "Acute changes of alpha-fetoprotein and human chorionic gonadotropin during induction chemotherapy of germ cell tumors". Cancer Res. 42 : 4855-4861.

	Read the instructions before use		For <i>in vitro</i> diagnostic use
	Temperature limitations		Do not reuse
	Manufacturer		



Manufactured by VEDALAB - France