VERAVAS
transforming laboratory medicine

VeraTest Biotin™

Part No. 500030

For Research Use Only

NAME OF THE PRODUCT

VeraTest Biotin

INTENDED USE

VeraTest Biotin is a colloidal gold based lateral flow assay to be used with the True Diagnostics™ Digital Reader for the qualitative determination of Biotin levels in serum or plasma samples.

The biotin lateral flow screening product is not intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae.

SUMMARY AND EXPLANATION

Biotin, also known as vitamin B7, is a water-soluble B vitamin often found in multi-vitamins and over the counter health and beauty supplements. *In vitro* laboratory tests that employ streptavidin-biotin binding mechanisms have the potential to be affected by high circulating biotin concentrations. Biotin can be attached through covalent bond to a variety of targets—from large antibodies to steroid hormones—with minimal effect on their specific non-covalent binding with avidin, streptavidin, or NeutrAvidin proteins. Therefore, biotin has been frequently used in the detection systems of immunoassays of different forms.

Immunoassays are generally categorized as either sandwich immunoassays (non-competitive) or competitive inhibition immunoassays. In general, streptavidin-biotin binding is used during assay incubation to couple biotinylated antibodies in sandwich immunoassays, or biotinylated antigens in competitive immunoassays, to streptavidin-coated surfaces. When a biological specimen contains excess biotin, the biotin competes with the biotinylated antibodies or antigens for binding to the streptavidin-coated surfaces, resulting in reduced capture of the biotinylated antibodies or antigens. Excess biotin produces falsely low results in sandwich immunoassays because the assay signal is directly proportional to the analyte concentration. Excess biotin in competitive immunoassays causes falsely elevated results because the assay signal is inversely proportional to the analyte concentration. Specific details of biotin interference have been extensively described in other publications.*(1-13)*

Normal circulating concentrations of biotin derived from the diet and normal metabolism are too low (< 1 ng/mL) to interfere with biotinylated immunoassays. However, ingestion of high-dose biotin supplements (e.g., 5 mg or higher) can result in significantly elevated blood concentrations that can interfere with commonly used biotinylated immunoassays. In certain medical conditions, extremely high biotin doses (e.g., 100 mg or higher) can result in serum or plasma biotin levels of > 1000 ng/mL.(14)

According to the FDA, biotin in blood or other samples taken from individuals who are ingesting high levels of biotin can cause falsely high or falsely low results in biotin-based immunoassays, depending on the design of the assay. (14)

Biotin interference thresholds differ widely among assays, even on a single platform. Tests with biotin interference thresholds < 51 ng/mL are considered high risk tests, or vulnerable immunometric and competitive methods.(1)

VeraTest Biotin is a competitive lateral flow assay for the qualitative determination of Biotin levels in serum or plasma samples that may be problematic in some avidin, streptavidin, or NeutrAvidin based assays.

PRINCIPLE OF THE TEST

VeraTest Biotin consists of a True Diagnostics™ Digital Reader (hereafter referred to as Reader), an assay buffer, and a chromatographic absorbent device that involves a biotin-binding protein that selectively detects biotin in serum or plasma samples with a high degree of sensitivity. The approximate run time is 5 minutes.

VeraTest Biotin is a competitive binding lateral flow assay in which biotin in a serum or plasma sample competes with an immobilized biotin derivative for limited binding sites of dye labeled biotin binding protein. By utilizing the binding affinity and specificity between biotin and the biotin binding protein, the qualitative test permits fast detection of biotin level in a serum or plasma sample.

After a test stick is inserted into the Reader, sample applied and followed by assay buffer onto the test stick, the serum or plasma sample undergoes vertical capillary filtration through the porous filtration system. When biotin is absent in the serum or plasma sample, unbound biotin binding protein-dye conjugate binds to the biotin derivative immobilized on the membrane, producing a saturated rose-pink color band in the Test Zone. Conversely, when biotin level is at or above 15 ng/mL, free biotin competes with the immobilized biotin derivative on the membrane by binding to the biotin binding protein-dye conjugate, forming a biotin-biotin binding protein-dye complex, preventing the development of a rose-pink color band. A biotin interfering threshold of 15 ng/mL is internally defined in the algorithm of the Reader. When the concentration of biotin is at or above 15 ng/mL, a rose-pink color band in the Test Zone with reduced saturation is expected. At the end of the run, the result will be measured and shown on the LCD display automatically in approximately 5 minutes. The results are presented as “YES +” or “NO -” by the Reader according to the level of saturation of the color band generated in the Test Zone.

REAGENTS AND MATERIALS PROVIDED

1. **20 - Test Sticks.** VeraTest Biotin test stick sealed in a protective foil pouch with desiccant. The reaction stick contains dye-conjugated and immobilized biotin binding protein and antibody in a protein matrix with sodium azide as a preservative.
2. **One - Qualitative Digital Reader.** Designed to work with 20 Test Sticks.
3. **Two - Vials of Assay Buffer.** 200 µL are used with each Test Stick. Contains sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipette, that accurately dispenses 20 µL and 200 µL
2. Disposable pipette tips
3. Personal protective equipment

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STORAGE AND STABILITY

The VeraTest Biotin may be stored at room temperature (15°-28°C) through its expiration date. Do not freeze or store at greater than 30°C. The product shelf life is approximately six (6) months. Refer to the expiration dates marked on the test stick *foil pouches* and assay buffer *vials* for stability of kit components.

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. Do not use test components beyond their expiration dates.
3. Do not use the Digital Reader beyond 20 test sticks.
4. This product contains sodium azide. For a specific listing, refer to the **REAGENTS AND MATERIALS PROVIDED** section. This material and its container must be disposed of in a safe way.
5. Dispose of all potentially contaminated test components in a biohazard container.
6. If specimens or test components have been stored in a refrigerator, allow them to warm to room temperature before performing the test.
7. When the test stick is ejected the display will turn off at 90 seconds.
8. A “YES +” test result indicates a sample contains biotin at or above the internal cut-off of the test (12.5 to 13 ng/mL). This could impact the results of biotin-sensitive diagnostic procedures. The Digital Reader will display “YES +” for 60 minutes before auto shut off.
9. A “NO -” test result indicates that suspect biotin levels are below the internal cut-off of the test (12.5 to 13 ng/mL). Please note that biotin levels could still be greater than 1 ng/mL. The Digital Reader will display “NO -” for 10 minutes before auto shut off.
10. If the internal control does not pass, then the result is invalid and a “?” will appear on the digital display. If the Digital Reader does not detect any sample flow within 10 minutes after insertion of the stick a “?” will appear on the digital display. The Digital Reader will display “?” for 10 minutes before auto shut off.

SPECIMENS COLLECTION AND PREPARATION

Follow manufactures specification for blood collection and serum or plasma preparation.

An internal matched sample-type study (serum, EDTA plasma, and Lithium Heparin plasma) study was completed to demonstrate equivalency by VeraTest Biotin.

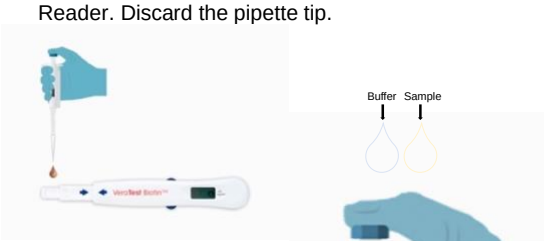
1. Seven (7) apparently healthy adult volunteers ingested 10 mg or 20 mg of over the counter (OTC) biotin, and had matched serum, EDTA plasma and Lithium Heparin plasma samples collected 4 hours post-biotin ingestion. All samples were aliquoted and stored frozen at -80° Celsius until testing.
2. Endogenous biotin levels in each sample were determined by LC-MS/MS. Biotin concentrations ranged from 12 to 48 ng/mL.
3. Samples were also tested by VeraTest Biotin.
4. All samples ≥ 15 ng/mL by LC-MS/MS (N=18) read "YES +" by VeraTest Biotin. All samples < 15 ng/mL by LC-MS/MS (N=3) read "NO -" by VeraTest Biotin.
5. For each Sample ID the matched sample types resulted in equivalent VeraTest Biotin results.

Sample ID	Sample Type	Biotin Dose (mg)	Biotin by LC-MS/MS (ng/mL)	VeraTest Biotin
1	Serum	10	12	NO -
	EDTA plasma		13	NO -
	LiHep plasma		12	NO -
2	Serum	20	40	YES +
	EDTA plasma		44	YES+
	LiHep plasma		45	YES +
3	Serum	10	19	YES +
	EDTA plasma		19	YES+
	LiHep plasma		19	YES +
4	Serum	20	31	YES +
	EDTA plasma		29	YES+
	LiHep plasma		31	YES +
5	Serum	10	23	YES +
	EDTA plasma		23	YES+
	LiHep plasma		21	YES +
6	Serum	20	48	YES +
	EDTA plasma		48	YES+
	LiHep plasma		47	YES +
7	Serum	10	24	YES +
	EDTA plasma		25	YES+
	LiHep plasma		23	YES +

ASSAY PROCEDURE:

1. Remove the VeraTest Biotin test stick from the foil pouch.
2. Insert the test stick into the qualitative Digital Reader cartridge port. This will turn the reader on and display the **steady** “Timer” symbol.

3. Place the Reader on a clean, level surface.
4. Before adding serum or plasma, ensure the “Timer” symbol on the display is still **steady.** If the timer is blinking, remove and reinsert the test stick.
5. Aspirate and dispense 20 µL of serum or plasma on to the test stick. The sample should be dispensed off-center in the portion of the sample pad closest to the Reader. Discard the pipette tip.



6. Before adding assay buffer, please ensure the “Timer” symbol on the display is still **steady**. If not, please take another test stick, and repeat from step 1.
7. Using a new pipette tip, aspirate and dispense 200 µL of the assay buffer on to the center of the sample pad.
8. The timer symbol will start to flash automatically ~ 45 seconds after adding the assay buffer.
9. After ~ 5 minutes the result will be displayed automatically.
10. Discard the test stick. Do not manually read a test result from the used test stick.

11. Discard the Digital Reader after 20 Test Sticks have been read.

INTERPRETATION OF RESULTS

"YES +"
A "YES +" result indicates biotin has been detected at or above 15 ng/mL in the sample specimen. The Digital Reader will display "YES +" for 60 minutes before auto shut off. When the test stick is ejected the display will turn off at 90 seconds.

"NO -"
A "NO -" result indicates that the concentration of biotin is below 15 ng/mL in the sample specimen. The Digital Reader will display "NO -" for 10 minutes before auto shut off. When the test stick is ejected the display will turn off at 90 seconds.

"?"
If the internal control does not pass, then the result is invalid and a "?" will appear. If the Digital Reader does not detect any sample flow within 10 minutes after insertion of the stick a "?" will appear. The Digital Reader will display "?" for 10 minutes before auto shut off. When the test stick is ejected the display will turn off at 90 seconds.

INTERNAL CONTROL
Each test stick has its own built-in control indicator. If, after performing the test, a "?" is reported on the qualitative Digital Reader, the device may have been under loaded with specimen or the test stick may have deteriorated. The assay will have to be repeated using a new test stick. Re-read the instructions carefully or call Veravas, Inc. for assistance.

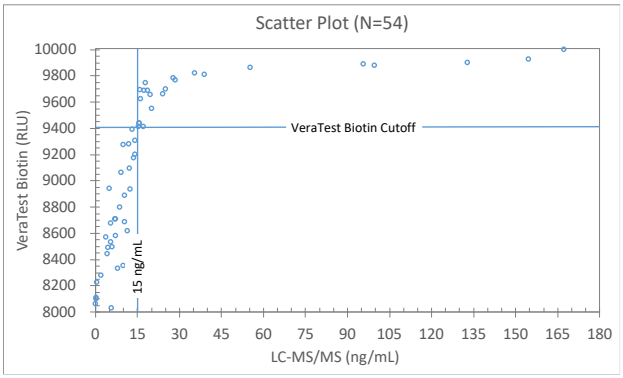
LIMITATION OF THE TEST

1. For Research Use Only. Not for use in diagnostic procedures.
2. A "YES +" or "NO -" result does not indicate that biotin interferes with the assay.
3. Heterophilic antibodies in human serum or plasma can react with reagent proteins and immunoglobulins. Individuals routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed.
4. Serum and plasma specimens may be infectious. Properly handle and dispose all used test sticks into an approved biohazard container. Residual specimens should be disposed in a medically approved manner after completion of all testing including the confirmatory testing.

ANALYTICAL PERFORMANCE

A small method comparison study was performed to demonstrate how VeraTest Biotin compares to LC-MS/MS.

1. The concentration of endogenous biotin in 54 serum samples was determined by LC-MS/MS. Biotin levels ranged from 0.18 ng/mL to 167 ng/mL, with the majority of the samples having values near the VeraTest Biotin cut-off of 15 ng/mL.
2. All samples ≥ 15 ng/mL by LC-MS/MS (N=23) read "YES +" by VeraTest Biotin. All samples < 15 ng/mL by LC-MS/MS (N=31) read "NO -" by VeraTest Biotin.



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