VIDAS® T3 (T3)

IVD

The VIDAS® T3 (T3) assay is intended for use on the instruments of the VIDAS family (VITEK® ImmunoDiagnostic Assay System) as an automated enzyme-linked fluorescent immunoassay for the quantitative determination of triiodothyronine (T3) concentration in serum or plasma (heparin). It is intended for use as an aid in the diagnosis and treatment of thyroid disorders such as hyperthyroidism.

SUMMARY AND EXPLANATION OF THE TEST

Triiodothyronine (T3) is a hormone that originates partially from direct thyroid secretion (approximately 20%), but mainly from peripheral conversion of T4 to T3 (approximately 80%). Since T3 has a greater physiological potency than T4, it contributes significantly to the maintenance of the euthyroid state (1,2).

T3 circulates in the bloodstream as a mixture of free and serum protein-bound hormone. The majority (> 99%) of T3 is bound to carrier proteins, primarily to thyroxine binding globulin (TBG) and to a lesser degree to thyroxine binding prealbumin (TBPA) and albumin. The free fraction (FT3) represents < 0.1% of the total T3 concentration and is the biologically active fraction (1,3).

T3 measurements are more sensitive to certain thyroid conditions than T4. Clinically, T3 blood levels better define hyperthyroidism and are especially valuable in following the course of therapy for this disorder. The T3 level is also a good indicator of the ability of the thyroid to respond to both stimulatory and suppressive tests. Under conditions of strong thyroid stimulation, the T3 concentration offers a good estimation of the thyroidal reserve (4,5).

There is, in general, a good correlation between T3 and T4 levels. However in some clinical situations, the T3 value can be disproportionately high with respect to the T4 level, for example in T3-thyrotoxicosis. In iodine deficiency, elevated TSH levels associated with low T4 concentrations are common and indicative of hypothyroidism, but when taken into consideration with a normal or slightly elevated T3 level, indicates euthyroidism in most patients (4,5).

T3 concentrations are altered by physiological or pathological changes in thyroxine binding protein (TBP) capacity. For example, factors such as estrogen, glucocorticoid or androgen therapy, pregnancy, oral contraceptives, nephrotic syndrome, and genetic influences can cause substantial changes in TBG levels (6,7).

Consequently, T3 concentrations in these situations may not accurately reflect thyroid status. Therefore, the accurate diagnosis of thyroid status should include other thyroid hormone tests such as TSH, T4, T Uptake or FT4 in conjunction with clinical evaluation.

PRINCIPLES OF THE PROCEDURE

The VIDAS T3 (T3) assay is an enzyme-linked fluorescent immunoassay (ELFA) that is performed in an automated instrument. All assay steps and assay temperature are controlled by the instrument. A pipette tip-like disposable device, the Solid Phase Receptacle (SPR®), serves as a solid phase for the assay as well as a pipetting device. At the time of manufacture, the SPRs are coated with sheep monoclonal anti-T3 antibodies. The VIDAS T3 (T3) assay configuration prevents nonspecific reactions with the SPR. Reagents for the assay are in the sealed T3 Reagent Strips.

The sample is transferred into the well containing the T3 antigen conjugated with alkaline phosphatase. The conjugate solution also contains ANS and sodium salicylate, which liberate bound T3 from the carrier proteins in the sample. The sample/conjugate mixture is cycled in and out of the SPR and the T3 in the sample competes with the T3-alkaline phosphatase conjugate for binding with the sheep monoclonal anti-T3 antibodies coated on the SPR. Wash steps remove unbound conjugate.

A fluorescent substrate, 4-methylumbelliferyl phosphate, is cycled through the SPR. Enzyme remaining on the SPR well will catalyze the conversion of the substrate to the fluorescent product 4-methylumbelliferone (450 nm). The intensity of fluorescence is measured by the optical scanner in the instrument; it is inversely proportional to the T3 concentration present in the sample.

When the VIDAS T3 (T3) assay is completed, the results are analyzed automatically by the instrument, and a report is printed for each sample.

KIT COMPOSITION (60 TESTS):

60 T3 Reagent Strips	STR	Ready-to-use.
60 T3 SPRs (2 x 30)	SPR®	Ready-to-use. SPRs are coated with sheep monoclonal anti-T3 antibodies.
T3 Control (liquid) (1 x 2 ml)	C1	Ready-to-use. Human serum* with T3 and 1g/L sodium azide. MLE data indicate the confidence interval in nmol/l ("Control C1 Dose Value Range").
T3 Calibrator (liquid) (1 x 2 ml)	S1	Ready-to-use. Human serum* with T3 and 1g/L sodium azide. MLE data indicate the concentration in nmol/l ("Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value" ("Calibrator (S1) RFV Range").
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit. or • MLE bar codes printed on the box label.		
1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

* This product has been tested and shown to be negative for HBs antigen, antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, the usual safety procedures should be observed when handling.

The SPR®

The interior of the SPR is coated during production with anti-T3 monoclonal antibodies (sheep). Each SPR is identified by the "T3" code. Only remove the required number of SPRs from the pouch and **carefully reseal the pouch after opening.**

The strip

The strip consists of 10 wells covered with a labeled, foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of the T3 Reagent Strip

Wells	Reagents
1	Sample well
2-3-4-5	Empty wells
6	Conjugate: A derivative of T3 antigen conjugated to alkaline phosphatase with ANS (0.95 mmol/l), sodium salicylate (11.9 mmol/l), and 1g/L sodium azide (400 µl)
7-8-9	Wash buffer: TRIS buffered saline (1 mmol/l, pH 7.4) with Tween 20 (0.05%) and 1g/L sodium azide (600 µl)
10	Reading cuvette with substrate: 4-methyl-ombelliferyl phosphate (0.6 mmol/l) + diethanolamine (DEA*) (0.62 mol/l or 6.6%, pH 9.2) + 1 g/l sodium azide (300 µl).

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Safety Data Sheet.

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipette with disposable tips that will dispense 100 µl.
- Powderless disposable gloves.
- For other specific materials, please refer to the Instrument Operator's Manual.
- Instrument of the VIDAS family.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.
For professional use only.

- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (see Laboratory biosafety manual - WHO - Geneva - latest edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Consider all patient specimens potentially infectious and observe routine biosafety precautions. Dispose of all used components and other contaminated materials by acceptable procedures for potentially biohazardous human blood products.
- Do not mix reagents or disposables from different lots.
- Kit reagents contain 1 g/L sodium azide which could react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- Powderless gloves are recommended as powder has been reported as a cause of false results in some enzyme immunoassays.
- The substrate (well 10) contains an irritant agent (diethanolamine*).* Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped thoroughly after treatment with liquid detergent and a solution of household bleach containing at least 0.5 % sodium hypochlorite to inactivate infectious agents. See the Operator's Manual for cleaning spills on or in the instrument. Do not place solutions containing bleach in the autoclave.
- The instrument should be routinely cleaned and decontaminated. See the Operator's Manual for the appropriate procedures.

STORAGE AND HANDLING

- Store the VIDAS® T3 (T3)Kit at 2-8ºC. **Do not freeze reagents.** Return unused components to 2-8ºC.
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain the stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label.

SPECIMEN COLLECTION AND PREPARATION

Acceptable specimens include serum or plasma (with heparin anticoagulant). Do not use plasma collected with EDTA. The use of heat inactivated sera has not been established with this test - do not heat sera. Samples can be stored at 2-8ºC in stoppered tubes for up to 2 days. If longer storage is required, the sera or plasma can be stored at -25 ± 6°C for up to 2 months. Avoid repeated cycles of freezing and thawing. If necessary, clarify samples by centrifugation.

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.
If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.
Note: the master lot data need only be entered once for each lot.
It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by "S1", must be tested **in triplicate** (see Operator's Manual). The calibration value must be within the set RFV (Relative Fluorescence Value). If this is not the case: recalibrate.

Assay Procedure

1. Remove necessary components from the kit and return all unused components to storage at 2-8ºC.
2. Allow components to reach room temperature (approximately 30 minutes).
3. Use one "T3" strip and one "T3" SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed.**
4. The test is identified by the "T3" code on the instrument. The calibrator must be identified by "S1", and tested **in triplicate**. If the control is to be tested, it should be identified by "C1".
5. If needed, label the "T3" Reagent Strips with the appropriate sample identification numbers.
6. Mix the calibrator, control, and sample using a vortex-type mixer (for serum or plasma separated from the pellet).
7. **For this test, the calibrator, control, and sample test portion is 100 µl.**
8. Insert the "T3" Reagent Strips and SPRs into appropriate positions on the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.

9. Initiate the assay processing as directed in the Operator's Manual. All steps will be executed automatically by the instrument.
10. Reclose the vials and return them to 2–8°C after pipetting.
11. The assay will be completed within approximately 40 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
12. Dispose of the used SPRs and strips into an appropriate recipient.

QUALITY CONTROL

A control is included in each VIDAS T3 (T3) kit. This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.
Results cannot be validated if the control value deviates from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

RESULTS AND INTERPRETATION

Two instrument readings for fluorescence in the Reagent Strip's reading cuvette are taken for each specimen tested. The first reading is a background reading of the cuvette and substrate before the SPR® is introduced into the substrate. The second reading is taken after the substrate has been exposed to the enzyme conjugate remaining on the interior of the SPR. The background reading is subtracted from the final reading to give a Relative Fluorescence Value (RFV) for the test result.
The range of results for the VIDAS® T3 (T3)assay is 0.4 - 9.0 nmol/l. Samples with results less than 0.4 nmol should be reported as "< 0.4 nmol/l". Samples with T3 concentrations greater than 9 nmol/l are reported as "> 9 nmol/l". Dilute these samples 1/2 (1 volume of sample + 1 volume of Control "C1" or one volume of normal serum) and retest in the VIDAS T3 (T3)assay. The result is first multiplied by two and then the concentration of C1 or of the normal serum used is subtracted to obtain the T3 concentration.

A report is printed which records :
- the type of test performed,
- the sample identification,
- the date and time,
- the lot number and the expiration date of the reagent kit being used,
- each sample's RFV and T3 concentration.

LIMITATIONS OF THE TEST

1. VIDAS T3 (T3) assay results should be used in conjunction with clinical symptoms, other thyroid test results, etc.
2. The use of neonatal specimens has not been established for the VIDAS T3 (T3)assay.

PERFORMANCE DATA

Immunological Specificity

The antibody used in the VIDAS T3 (T3) assay was tested for cross-reactivity against a number of compounds. The results in the table below are represented as the percentage ratio between the T3 concentration and the cross reactant concentration at 50 % binding.

Tested compound	Cross-reactivity percentage
L-thyroxine free acid	0.21 %
D-thyroxine free acid	0.04 %
L-Triiodothyronine free acid	100 %
D-Triiodothyronine free acid	100 %
Diiodo-L-thyronine	3.3 %
Monoiodotyrosine	< 0.01 %
Diiodotyrosine	< 0.01 %
Diphenylhydantoïn	< 0.01 %
Propylthiouracil Anhydre	< 0.01 %
Triiodothyroacidic acid	100 %
Phenylbutazone crystalline	< 0.01%
Triiodothyropropionic acid	100 %
Sodium salicylate	< 0.01 %
Propionic acid	50 %

Detection limit

The VIDAS® T3 (T3) assay is designed to measure T3 levels between 0.4 and 9.0 nmol/l. The lowest measurable level of T3 (assay sensitivity) that can be distinguished from zero with 95 % probability using the VIDAS T3 (T3) assay is 0.05 nmol/l.

PRECISION/REPRODUCIBILITY

Intra-assay precision

Five serum samples were tested for intra-assay precision. Thirty replicates of each sample were tested in the same run.

Sample	1	2	3	4	5
Mean concentration (nmol/l)	0.68	1.80	3.16	6.17	8.71
% CV	11.0	5.3	4.4	2.7	2.3

Inter-assay reproducibility on the same instrument

Five serum samples were tested in 29 runs on the same instrument over a 8-week period (recalibration was performed every 14 days as described in the Operator's Manual).

Sample	1	2	3	4	5
Mean concentration (nmol/l)	0.76	1.93	3.34	5.84	6.42
% CV	12.4	5.7	4.2	2.8	3.2

Inter-instrument inter-assay reproducibility

Five serum samples were tested in singlet in 10 runs on different instruments.

Sample	1	2	3	4	5
Mean concentration (nmol/l)	0.65	1.77	3.19	6.27	8.55
% CV	7.3	6.3	6.1	3.9	1.6

PARALLELISM (Dilution Tests)

Three hyperthyroid samples were each diluted in sera with normal T3 concentrations and tested in singlet in 3 runs. The measured mean values compared to the expected mean values are shown as the mean recovery percentages in the table below.

Sample	Dilution factor	Expected values (nmol/l)	Measured values (nmol/l)	Mean recovery percentage (%)
1	1/1	6.33	6.33	100
	1/2	4.07	4.44	109
	1/4	2.93	3.13	107
	1/8	2.37	2.57	109
2	1/1	7.53	7.53	100
	1/2	4.53	5.22	115
	1/4	3.02	3.63	120
	1/8	2.27	2.55	112
3	1/1	3.99	3.99	100
	1/2	2.76	2.90	105
	1/4	2.14	2.15	101
	1/8	1.83	1.83	100

RECOVERY TESTS

Three samples were spiked with known quantities of T3 and tested in singlet in 3 runs. The measured mean concentration compared to the expected mean concentration is shown below.

Sample	Expected mean concentration (nmol/l)	Measured mean concentration (nmol/l)	Mean recovery percentage (%)
1	1.38	1.38	100
	2.32	2.39	103
	3.08	3.34	109
	3.49	3.98	114
	4.23	4.88	116
2	0.96	0.96	100
	2.11	2.11	100
	2.87	3.07	107
	3.28	3.92	120
	4.02	5.08	126
3	1.38	1.38	100
	2.32	2.39	103
	3.08	3.19	104
	3.49	3.89	111
	4.23	4.92	116

INTERFERENCE STUDIES

Method of Collection

Blood samples were collected from thirty patients. For each patient, four specimens were collected at the same time: in a dry glass tube; in a tube with separating gel; in a tube with silicone, and in a lithium heparinized tube, respectively. Each sample collected was tested in duplicate and sera from the same donor were tested in the same run. The dry glass tube was the reference to which the other methods were compared. The statistical ratio method that was used to evaluate the data showed that there was no significant difference with any of the specimen collection devices tested.

Heparin

Three pools of human sera were spiked with increasing quantities of lithium heparin.

		Amount of lithium heparin spiked (IU/ml)			
		0	0.5	5	50
T3 (nmol/l)	Pool 1	0.57	0.62	0.57	0.59
	Pool 2	3.07	3.16	3.31	3.05
	Pool 3	8.79	8.57	8.47	8.44

These data indicate that lithium heparin plasma may be used in the VIDAS® T3 (T3) assay.

EDTA

Three pools of human sera were spiked with increasing quantities of EDTA.

		Amount of EDTA spiked (mg/ml)			
		0	1	5	10
T3 (nmol/l)	Pool 1	0.66	0.67	1.55	2.72
	Pool 2	3.15	3.32	4.73	6.66
	Pool 3	8.36	8.83	9.00	9.00

These data show an increase in values. Do not use EDTA plasma in the VIDAS T3 (T3) assay.

Hemoglobin

Two pools of human sera were spiked with increasing quantities of hemoglobin.

		Amount of hemoglobin spiked (µmol/l)						
		0	15	30	60	150	186	300
T3 (nmol/l)	Pool 1	1.65	1.69	1.70	1.63	1.74	1.57	1.60
	Pool 2	6.21	6.04	5.97	6.05	5.97	5.93	5.76

Lipids

Two pools of human sera were spiked with increasing quantities of a lipid solution.

		Amount of triglycerides spiked (g/l)				
		0	0.25	0.5	1.0	2.0
T3 (nmol/l)	Pool 1	1.61	1.52	1.55	1.60	1.82
	Pool 2	6.10	6.11	6.16	6.20	6.05
Appearance		Clear	Opalescent	Turbid		

Bilirubin

Two pools of human sera were spiked with increasing quantities of bilirubin.

		Amount of bilirubin spiked (µmol/l)						
		0	15	30	70	145	230	320
T3 (nmol/l)	Pool 1	1.49	1.39	1.40	1.39	1.45	1.40	1.31
	Pool 2	1.39	1.44	1.47	1.55	1.45	1.44	1.51

Although interference linked to the presence of hemoglobin, bilirubin or lipids has not been observed, using hemolyzed, icteric or lipemic samples is not recommended. If possible, collect a new specimen.

EXPECTED VALUES

A normal range study was performed using a total of 148 samples from euthyroid patients. The results from testing these samples in the VIDAS® T3 (T3) assay showed that 99% of the values ranged from 0.92 nmol/l to 2.33 nmol/l.
Conversion factors :

- From nmol/l to µg/dl multiply by 0.0651
- From nmol/l to µg/l multiply by 0.651
- From nmol/l to ng/dl multiply by 65.1

CORRELATIONS

Serum and plasma (heparin) samples with T3 concentrations ranging from 0.4 nmol/l to 9 nmol/l were tested using the VIDAS T3 (T3) assay and commercially available T3 EIA and RIA tests. The results of the correlations are shown below:

	Number of Samples	Equation of the Line	Correlation coefficient
EIA	164	y = 1.02x + 0.04	0.94
RIA	199	y = 1.01x + 0.35	0.95

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user
Note:	*Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	13697C	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	KIT COMPOSITION (60 tests) WARNINGS AND PRECAUTIONS
2015/06	13697D	Technical	KIT COMPOSITION (60 tests) INSTRUCTIONS FOR USE

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