

Use of the “Veda-Lab Easy Reader+” for the Determination of T3, T4 and TSH Hormones in the Mountainous Population of Benin: Case of Natitingou

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Abstract

The thyroid, an endocrine gland located at the base of the neck, produces thyroid hormones (triiodothyronine (T3) and thyroxine (T4)). The production of these hormones is possible by iodine and other nutrients such as selenium and certain vitamins. To assess the thyroid disturbances in the mountain population of Benin, a survey was conducted in Natitingou, a mountain town located in the department of Atacora, in the northwest of Benin, on a sample of thirty (30) adults (15 men and 15 women), most of whom are educated. The results of the questionnaire revealed that 43% of the surveyed population acknowledged having knowledge on the mentioned subject and have dietary habits based on the consumption of seafood, and also legumes (20%). The examination of the serum results of the dosage of T3, T4 and TSH hormones revealed cases of thyroid disturbances in the region (36.32% in men and 44.98% in women). The analysis of a comparative table including the “VEDALAB Easy Reader+” and five (05) other readers, presents the performance, reading techniques, principles, advantages and disadvantages of each device. Pending further studies, some recommendations were made at the end of this study to the academic authorities regarding probable cases of dysthyroidism for which additional examinations are required and an awareness for the improvement of dietary habits.

Keywords

Thyroid, TSH, T3, T4, “VEDALAB Easy Reader+”

1. Introduction

The thyroid is an endocrine gland whose mechanism of action is better known nowadays. It is a gland of about 5 cm in diameter located under the skin of the neck and below the Adam's apple [1]. The two halves (lobes) of the gland are connected by a central part (called the isthmus) that gives the thyroid its butterfly shape. Normally, the thyroid is not visible and can barely be felt. If it increases in size, doctors may feel it or see it as a lump underneath or to the side of the Adam's apple. The thyroid secretes hormones that control the rate of chemical functions in the body. These thyroid hormones are: Thyroxine (T₄) also called Tetraiodothyronine and Triiodothyronine (T₃). To make these hormones, the thyroid needs iodine, an element found in water and food. Thyroid disease is one of the most common hormonal disorders seen in our society today [1], the most common forms being hyperthyroidism, hypothyroidism and thyroid nodules. Iodine is an essential micronutrient for the body because of its important role in the production of thyroid hormones [2]. Its intake is essentially dietary and the daily needs of an adult are estimated at 150 µg [3]). It is also involved in the metabolism of carbohydrates, lipids and proteins. Adequate iodine intake from a young age is necessary to prevent the onset of severe pathologies such as cretinism associated with iodine deficiency [4]. Iodine deficiency, for example, inhibits growth and can cause thyroid disorders. Worldwide, it is estimated that more than 2.2 billion people are affected, *i.e.* 38% of the population, including 30% to 70% of goiter cases and 1% to 10% of cretinism [5]. In Benin, as elsewhere, the majority of iodine is of marine origin. It is present in large quantities in iodized sea salt. All sea products are good sources of iodine. Due to precipitation, it is found in the land and in the plants we eat. Iodine measurement in some water sources in the hill department (a region far from the southern part where the sea is located) shows a decrease in iodine level (44 µg/L) compared to the normal level of 150 µg/L according to WHO [6] [7] [8]. The soil, according to the studies, is poor in iodine and the inhabitants with a low ioduria level are subject to a permanent disturbance of the thyroid gland function. The city of Natitingou, a region even further south and close to Tanguiéta (a region whose soil is also poor in iodine), still has no data on the prevalence of thyroid disorders. It is to fill this gap that the theme “*Use of the ‘Veda-Lab Easy Reader+’ for the determination of T₃, T₄ and TSH hormones, parameters for the assessment of thyroid disorders, in adults in mountainous areas of Benin: the case of the city of Natitingou*” was chosen. The **Veda-Lab Easy Reader+** was chosen for the determination of biological parameters because of its availability, affordability and ease of use. The results of this survey will give an idea of the state of thyroid disorders in Natitingou in order to formulate subsequent recommendations to correct these facts through a review of eating habits.

In view of previous studies conducted in the commune of Tanguiéta on the prevalence of thyroid disorders and the lack of preliminary data on the subject in the sister commune of Natitingou, it is essential to conduct a survey in the three

central districts of this commune to present the current situation using the “*Veda-Lab Easy Reader+*” for the determination of T3, T4 and TSH hormones.

2. Materials and Methods

2.1. Sampling

For our study, we needed people with different roles. These were: 1) An agent who presented the project to the population in order to obtain their agreement to participate in the work and to help those who could not fill in the survey form; 2) A health agent to carry out the different blood samples. A non-probability sampling technique involving non-random selection based on convenience was performed to collect a total of 30 blood samples from adults of both sexes.

2.2. The Survey Form

As part of the work and to have more information about our volunteers, it was designed a survey form that allowed us to have information related to: age, gender, dietary habits, previous knowledge about thyroid, iodine and their roles.

2.3. Measuring Equipment

In order to reach the objectives, we have set, TSH, T3 and T4 determinations must be performed. To do this in good conditions, certain materials and consumables are essential. We can mention: The VEDA LAB *Easy reader* which is a quantitative reader of rapid tests, the tube centrifuge, the VEDA LAB TSH, T3 and T4 cassettes, in boxes containing everything necessary for twenty tests. These are not only the cassettes, but also disposable plastic pipettes, diluents, instructions for use and positive and negative controls.

3. Methods

For data collection, we proceeded as follows: Prior to the data collection, an individual interview to explain the motivations of this survey and its different steps. Filling out the individual survey form containing information such as age, types of food consumed frequently, information about the patient's iodine and thyroid hormones, etc...All the ethical issues of this study have been taken into account as in the study of SEZAN and al [6] [7] [8]. The measure the TSH, T3 and T4 hormone levels on a VEDALAB cassette reader. The reading technique and principle focused on immunochromatography and based on the unique combination of colloidal gold-labeled monoclonal antibodies and polyclonal antibodies on solid phase. As the sample migrates on the absorbent paper, the colloidal gold-labeled conjugate binds to the desired element forming an antigen-antibody complex. This complex binds to the solid phase antibodies in the T reaction zone producing a pink band. In the absence of the desired element, no band appears in the T reading zone. The mixture continues its migration along the membrane. The free conjugate is then immobilized in the control zone C to give a pink band indicating that the test has been performed correctly.

4. Results

As part of our research, we compared several methods of measuring hormones, conducted a survey to get an idea of the dietary habits of individuals and then performed biological tests with the VEDA LAB *Easy reader* to identify cases of thyroid disruption.

4.1. Results of the Survey

Figure 1 presents the distribution of volunteers by gender. At the pre-survey, thirty volunteers completed the individual survey form before agreeing to be sampled. An equal number of females and males between the ages of 22 and 50 were sampled, 15 per gender, and their ages ranged from 22 to 50 years.

Figure 2 shows the distribution of individuals based on their seafood eating habits. After the analysis of the survey forms, we were able to get an idea of the eating habits of thirty individuals who were the subject of our work.

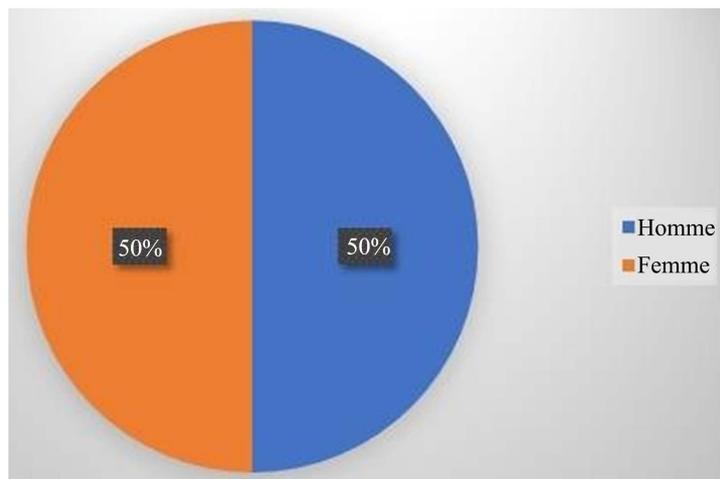


Figure 1. Pie chart representing the distribution of volunteers by gender.

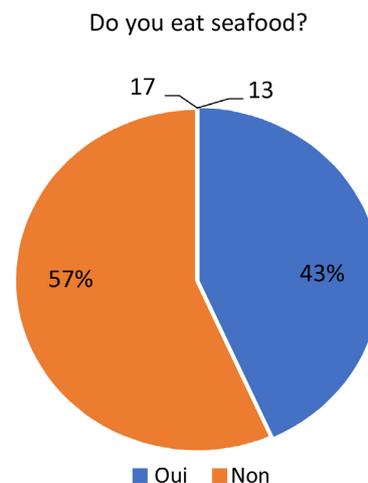


Figure 2. Pie chart showing the distribution of individuals based on whether or not they eat seafood.

When asking what foods they often eat, several responses were given. **Table 1** presents the distribution of individuals according to the foods commonly consumed.

In summary, the results of the questionnaire revealed that 43% of the surveyed population recognized that they had knowledge on the subject mentioned and had eating habits based on the consumption of seafood, and also legumes (20%).

4.2. Results of Biological Examinations

After the manipulations, the following results were obtained and have been grouped in the **Table 2** and **Table 3**.

From the results of **Table 2** and **Table 3**, we note the presence of thyroid disturbances in the individuals sampled as following:

Triiodothyronine T3:

13.33% of males and **20%** of females are below normal

13.33% of men and **13.33%** of women have a high score compared to normal

Table 1. Distribution of individuals according to the foods commonly consumed.

Food groups	Number (30)	Percentage (%)
Cereals	24	80
Vegetables	3	10
Bean	6	20

Table 2. T3, T4 and TSH results in men.

Order	T3 (ng/ml)	T4 (µg/dl)	TSH (mIU/L)
	Standard: 0.7 - 2 ng/ml	Standard: 4 - 11 µg/dl	0.5 - 5 mIU/L
1	5.1	4.1	2.2
2	<0.3	5.6	5
3	<0.3	2.8	<2
4	0.8	7.9	<2
5	1.2	2	3
6	0.7	5.3	<2
7	1	3.1	6.2
8	1	1.9	10.7
9	1.2	4.6	4.5
10	0.8	4.8	3.8
11	1.2	4	3.6
12	4.9	2.4	<2
13	0.8	4.6	3.1
14	0.9	2.5	5
15	0.8	4.5	<2

Table 3. T3, T4 and TSH results in women.

Order	T3 (ng/ml)	T4 (µg/dl)	TSH (mIU/L)
	Standard: 0.7 - 2 ng/ml	Standard: 4 - 11 µg/dl	0.5 - 5 mIU/L
1	2	3.4	22.1
2	0.5	2	5.6
3	0.9	6.3	<2
4	0.8	8.1	<2
5	0.7	7.1	<2
6	0.8	5.2	<2
7	<0.3	8.2	<2
8	4.1	8.1	<2
9	2.4	12.8	<2
10	1.3	3.1	<2
11	0.6	1.9	<2
12	1.9	1.8	5.3
13	0.7	1.7	2.8
14	1.9	4.7	3
15	1.7	2.5	<2

Thyroxine T4:

40% of men and 33.33% of women have a result below the norm

0% of men and 6.66% of women have an elevated T4 result

Thyroid Stimulating Hormone TSH:

40% of men and 75% of women have a TSH result below 2

20% of men and 20% of women have an elevated TSH result

In sum 26.66% of men and 33.33% of women have abnormal T3 results, 40% of men and 40% of women have abnormal T4 results. Regarding TSH, 20% of individuals of each sex have high results.

For a proper and useful interpretation of the thyroid hormone results, it is essential to consider all results simultaneously.

Indeed two men out of fifteen (2/15) or 3% met the conditions of hypothyroidism with high TSH values and low T4 values with or without T3 (Table 2). Three women out of fifteen (3/15), or 5%, met the conditions for hypothyroidism with high TSH values and low T4 values with or without T3 (Table 3). Some individuals have low T4 values with low or normal T3 and have normal or low TSH values. These individuals represent 20% of men and 26.66% of women. These normal or low TSH values could be explained by the presence of pituitary insufficiency. These are cases of hypothyroidism probably due to pituitary insufficiency.

One male individual (1/15) or 6.66% of males has low T4, high T3 and low or normal TSH values. One male (1/15) or 6.66% and one female (1/15) or 6.66% had normal T4, high T3 and normal or low TSH values. One woman (1/15) or

6.66% has high T3 and T4 values with normal or low TSH values. These cases are encountered in pauci symptomatic hyperthyroidism.

In sum, 23% of men have hypothyroidism of which 20% are probably due to pituitary insufficiency.

31.66% of women have hypothyroidism of which 26.66% could be due to pituitary insufficiency.

13.32% of men 13.32% of women have hypothyroidism that is probably pauci symptomatic.

Figures 3-5 are the histogram showing the number of high, low and normal T3, T4 and TSH results within both sexes respectively.

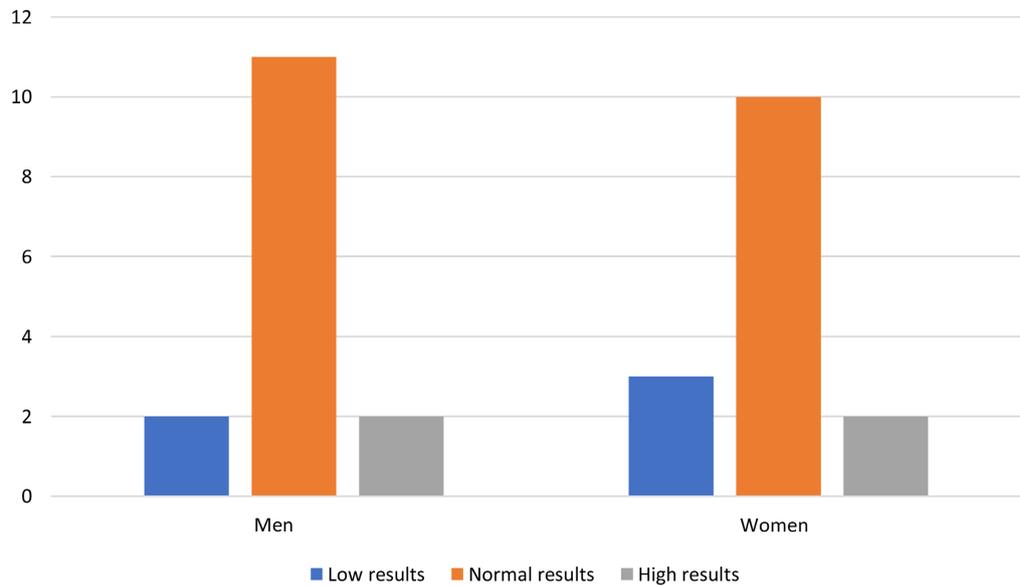


Figure 3. Histogram showing the number of high, low and normal T3 results within both sexes.

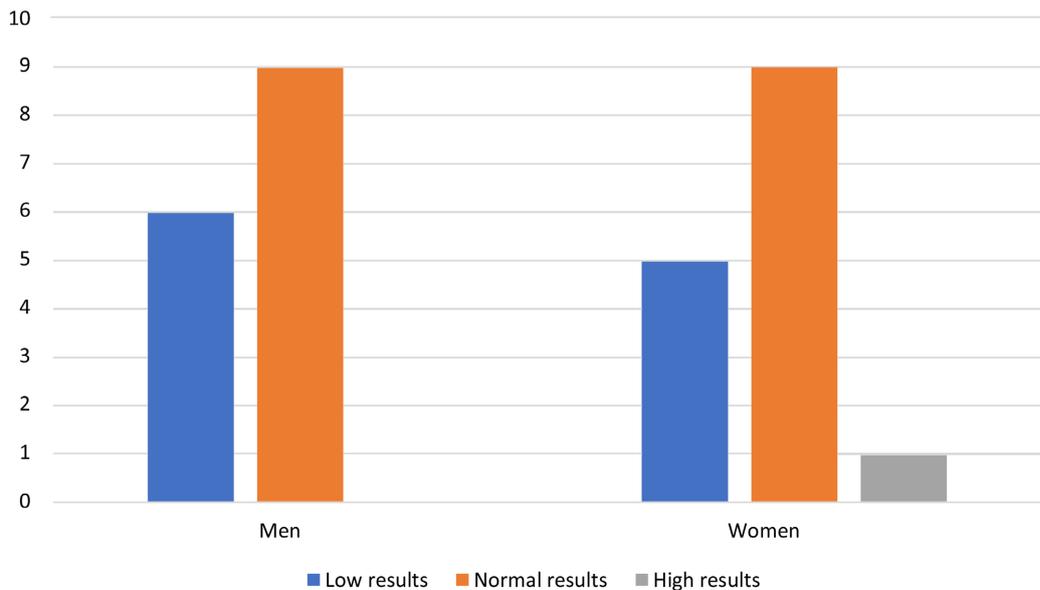


Figure 4. Histogram showing the number of high, low and normal T4 results within both sexes.

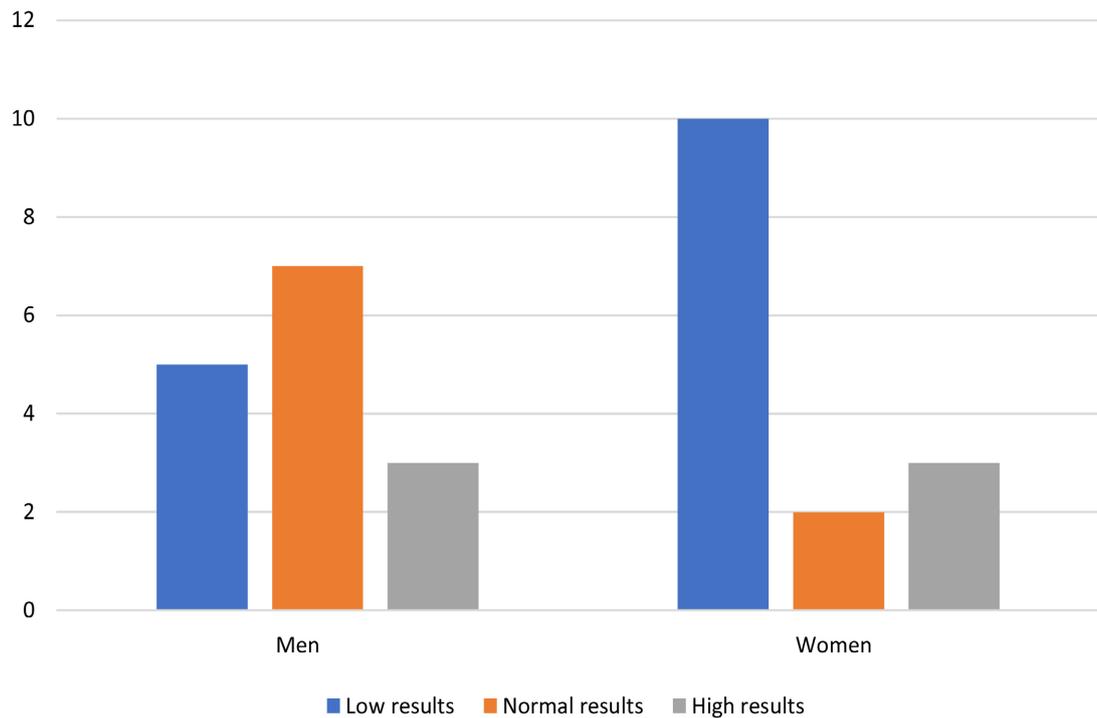


Figure 5. Histogram showing the number of high, low and normal TSH results within both sexes.

In summary, the examination of serum results of T3, T4 and TSH hormones revealed cases of thyroid disturbances in the region (36.32% in men and 44.98% in women).

In **Table 4**, we compare the technical features and specifications (performances, reading techniques, principles, advantages and disadvantages) of the “**VEDALAB Easy Reader+**” to five other readers for determination of T3, T4 and TSH hormones.

It was seen from **Table 4** that the devices: 1) ELISA reader; 2) Architect i 1000 sr; 3) Finecare read within the wavelengths 340 to 700 nm; 400 to 700 nm and 470 to 525 nm respectively whereas MINI VIDAS and the Getein 1100 have Analytical module with 12 test positions/2 independent compartments of 6 tests and One-step test with 3 - 15 min per test, 5 sec/test for multiple tests respectively. The “**VEDALAB Easy Reader+**” is a reflectometer using a non-destructive analytical technique based on the reflection of light by surfaces and interfaces to measure characteristics such as color intensity and refractive index or some blood parameter concentrations. It is a quantitative lateral flow immunochromatography analyzer. Its installation kit includes the analyzer and the software that lets the operator actuate the evaluation processes, regulate the functioning, storing and moving measurement results database [9]. It is intended to provide a quantitative immunochromatography assay result for a wide range of otherwise purely qualitative rapid test devices to increase the accuracy and efficiency of the home, point of care, and laboratory testing of the relevant marker analytes.

Table 4. Comparison of the technical features and specifications (performances, reading techniques, principles, advantages and disadvantages) of the “VEDALAB Easy Reader+” with five other readers for determination of T3, T4 and TSH hormones.

N°	Devices	Technical specifications	Reading techniques	Principle	Benefits	Disadvantages
01	ELISA reader	Wavelength: 400 to 700 nm and 340 to 700 nm for those working in the ultraviolet.	Enzyme Immunoassay	Detection of an antibody or antigen captured on a solid surface using direct or secondary labeled antibody. This results in an enzymatic reaction whose product can be read with a spectrophotometer.	<ul style="list-style-type: none"> ➤ Very sensitive device. ➤ Specific device. ➤ Ability to perform a large number of tests at the same time. 	<ul style="list-style-type: none"> ➤ Relatively higher costs when small numbers of samples are involved. ➤ The slightest trace of dirt can bias the results. ➤ Longer handling time. ➤ The enzymatic reaction makes the technique temperature dependent.
02	MINI VIDAS	Analytical module with 12 test positions/2 independent compartments of 6 tests. Simultaneous processing of different parameters. No inter-reagent or inter-sample contamination. Up to 36 tests per hour.	Immunofluorescence	Based on ELFA (Enzyme Linked Fluorescent Assay) technology. Detection of an antibody or antigen captured on a solid surface using direct or secondary labeled antibody. This results in a fluorescent reaction whose product can be read with a spectrophotometer.	<ul style="list-style-type: none"> ➤ Sturdy and resistant device in spite of the duration. ➤ Sensitive and specific device ➤ Ability to handle multiple samples at once. 	<ul style="list-style-type: none"> ➤ Higher reading time than fast readers. ➤ Results very dependent on the temperature of the room. ➤ High cost of equipment and cartridges. ➤ Boxes not always available in Benin.
03	Architect i1000sr	Wavelength: 400 to 700 nm Up to 100 tests/hour Up to 50,000 stored results. Equipped with an automatic dilution system. Potential for contamination of the sample needle ≤ 0.1 parts per million. Stability of reagents on board: 14 to 30 days. Ability to detect samples, clots or bubbles. Possibility to control the pressure of the reagents Possibility of measuring the interference of the sample. Equipped with a PC with touch screen, keyboard and mouse. Robotic sample transfer system (RSH).	Chemiflex	Architect i1000sr meets high laboratory standards by providing STAT results with a flexible protocol that improves workflow and allows results to be reported with confidence.	<ul style="list-style-type: none"> ➤ Up to one hundred (100) tests per hour. ➤ Used for several types of samples (Serum, Plasma, Whole Blood, Urine). ➤ Possibility of automatic dilution. ➤ Clot and bubble detection capability. ➤ Online help available. 	<ul style="list-style-type: none"> ➤ High cost of equipment and reagents.

Continued

04	VEDALAB EASY Reader+	<p>Adaptable system: multilingual. Software update via internet.</p> <p>Usable with batteries.</p> <p>Profitability: reasonable price, currently usable on more than 36 parameters...</p>	Immunochromatography	<p>Based on the reflection of light by surfaces and interfaces to measure characteristics such as color intensity and refractive index or some blood parameter concentrations (Reflecto pho tometry). The method is based on the unique combination of colloidal gold-labeled monoclonal antibodies and polyclonal antibodies on solid phase. As the sample migrates on the absorbent paper, the colloidal gold-labeled conjugate binds to the desired element forming an antigen-antibody complex. This complex binds to the solid phase antibodies in the T reaction zone producing a pink band. In the absence of the desired element, no band appears in the T reading zone. The mixture continues its migration along the membrane. The free conjugate is then immobilized in the control zone C to give a pink band indicating that the test has been performed correctly.</p>	<ul style="list-style-type: none"> ➤ Lightweight and easy to carry. ➤ Relatively low cost of cassettes. ➤ Low influence of temperature on the results. ➤ Longer time to handle a large number of samples. ➤ Impossibility to manipulate certain parameters such as toxoplasmosis and rubella.
05	Getein 1100	<p>Data storage: 10,000 data (Max)</p> <p>Advanced fluorescence immunoassay with multiple quality control. One-step test with 3 - 15 min per test, 5 sec/test for multiple tests.</p> <p>RFID card calibration, keyboard and mouse connectivity via USB port, handwritten input now available, continuous testing for 3 hours with a lithium battery.</p>	Immunofluorescence	<p>The biological fluid is transferred to the appropriate test strip (test reagent). This test strip is inserted into the test channel of the Getein 1100 immuno-fluorescent analyzer and after 3 to 15 minutes, depending on the element tested, the test result is displayed on its 8" LCD touch screen.</p>	<ul style="list-style-type: none"> ➤ Fast results with the possibility of handling one or few samples in record time. ➤ Low influence of temperature on the results. ➤ Lightweight and easy to carry ➤ Relatively low cost of playback cassettes. ➤ Longer time to handle a large number of samples. ➤ Impossibility to manipulate certain parameters such as toxoplasmosis and rubella.

Continued

06	Finicare	<p>Reading between 470 and 525 nm. Good correlation with the CLIA method. Results available in 3 to 15 minutes. Direct connection to LIS/HIS</p> <p>Automatic reading of batch number and calibration information in the identification chip. Sampler specially designed for small specimen volumes</p>	Immunochromatography	<p>The biological fluid is transferred to the appropriate test strip (test reagent). This test strip is inserted into the test channel of the Finicare FIA Meter Plus (FS-113) and after 3 to 15 minutes, depending on the item being tested, the test result is displayed on the 8" LCD touch screen.</p>	<ul style="list-style-type: none"> ➤ Quick results. ➤ Ability to handle one or few samples in a short time (3 to 5 minutes per sample). ➤ Reliability of results is not dependent on temperature. ➤ Lightweight device, therefore easy to carry. 	<ul style="list-style-type: none"> ➤ Only one sample to be handled at a time. ➤ Inability to manipulate certain parameters such as toxoplasmosis and rubella.
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5. Discussions

In view of the results obtained from the questionnaire-based survey, we note a lack of information regarding the importance of seafood consumption, which is the best reservoir of iodine. Although they said they knew about and consumed seafood, none of the thirty (30) mentioned seafood as one of the most consumed foods. The majority of the individuals consumed cereals which contain small amounts of iodine. In view of the results obtained, we note the presence of thyroid disturbances in the individuals sampled. The point gives us:

The results of the serum dosage of T3, T4 and TSH hormones in this survey are similar to those obtained by SEZAN *et al.* (32% and 46% respectively) on thyroid disorders in the commune of Tanguiéta (commune located 48 km north of Natitingou).

The examination of the results of the “**VEDALAB Easy Reader+**” shows that this equipment, although powerful and financially very accessible, has its limits. Indeed, this device quantifies TSH concentrations between 2 and 80 mIU/l and does not present any inconvenience when TSH must be quantified to confirm cases of hypothyroidism. However, for the confirmation of hyperthyroidism cases in which the TSH concentration < 0.1 mIU/l, another reader should be used. The limitations of the “**VEDALAB Easy Reader+**” are explained by its technical specifications and operating principle. According to the comparison table, the ELISA microplate readers, the Mini VIDAS and the Architect i1000sr are the most sensitive but the cost of these testers and the cost of the reagents are higher than those of other rapid test readers. These readers are designed to handle high numbers of samples: up to 96 samples per hour for the ELISA, 12 for the Mini VIDAS and up to 500 per hour for the Architect i1000sr. Among the other rapid test readers, the VEDALAB Easy reader + was chosen because of its easy portability and the possibility to use it in the absence of electrical power. The availability of reagents and their affordable cost also contributed to the choice of this reader.

As limitation of use, any reactive specimen with any of the rapid test devices must be confirmed with alternative testing methods and clinical findings. The results obtained with rapid test devices should only be interpreted in conjunction with other diagnostic procedures and clinical findings. A negative result for an individual subject indicates that the level of the marker tested is not detectable. However, a negative test result does not preclude the possibility of one or more of the medical conditions associated with the given marker. As with other photometers, the main elements of reflectometers include a light source, usually long-life LEDs of specific wavelengths that are focused onto a sample surface via a lens system and the reflected light is measured by detectors.

6. Conclusion

The results of the survey conducted in Natitingou (a mountainous town located in the Atacora department, in the northwest of Benin) for the assessment of thyroid disturbances in a fringe of the population of this town support and confirm those obtained by SEZAN *et al.* in Tanguiéta, a sister and neighboring town. These results also show that the “**VEDALAB Easy Reader+**”, although powerful and financially very accessible, has reading limitations. Indeed, this device quantifies TSH concentrations between 2 and 80 mUI/l and does not present any disadvantage when TSH must be quantified to confirm cases of hypothyroidism. On the other hand, for the confirmation of hyperthyroidism cases in which the TSH concentration < 0.1 mIU/l, it is necessary to use other readers (ELISA, Mini VIDAS, Architect i1000, ...). The limitations of the “**VEDALAB Easy Reader+**” are explained by its technical specifications and operating principle.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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