Carci Reagent

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Detecting the concentration of tyrosine in urine with high performance liquid chromatography (HPLC)

（Experiment Test）（This test is done according to EN13612:2002）

No.: DCT-HPLC/ZYB/2021

Introduction

This test describes the research method of using high performance liquid chromatography to detect the concentration of tyrosine in urine. We used total of 110 samples (Cancer patients 45 cases -Liver cancer, Stomach cancer, Nasopharyngeal carcinoma, Lung cancer, Esophageal cancer, Cervical cancer, Lymphoma, Other cancers; Non-cancer patients 46 cases -tuberculosis, pneumonia, hepatitis, liver cirrhosis, and gastritis; Healthy people 19 cases). The purpose was to proof if urinary tyrosine concentration of cancer patients is high.

Content:

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1, Name of in vitro diagnostic device for self-testing

CarciReagent

2, Description of in vitro diagnostic device for self-testing

Test kit for the detection of the approximate amount of monohydroxyphenol metabolites (tyrosine) in urine (Lay semi-quantitative test for self-testing) (Chemical chromogenic method)

3, Intended use of the in vitro diagnostic device for self-testing

CarciReagent - an in vitro diagnostic device for self-testing, intended for the detection of monohydroxyphenol metabolites (tyrosine) and its approximate amount in the patient's urine (Lay semiquantitative test)

4, Principle and function of in vitro diagnostic device for self-testing

The basic principle of the test is based on the improved method of Millon's reagent (Millon's reagent), which monitors the increased amount of tyrosine (monohydric phenolic amino acids and their metabolites) in the urine. By changing the color of the mixture in the ampoule after adding 3 ml of morning urine (middle urine stream), the reaction color cascade can be used to determine if urine samples contain increased amounts of these metabolites. The reagents in the ampoule and the tyrosine content in the urine show a characteristic chromogenic response that can be used for the clinical diagnosis of intracellular metabolic abnormalities (detection of possible changes or disorders in metabolism within the human cell). The determined approximate content of tyrosine in urine (according to the attached table from 0–2000 mg per liter of urine) reacts with the chemical reagent and, depending on its amount, turns colored. According to the enclosed color scale, the test result can be read from No. 1 to No. 8. The result from No. 1 to No. 3 is the amount of tyrosine in the normal concentration, so the result is considered negative, and no increased amount of tyrosine has been demonstrated. With results No. 4 and No. 5, it is already a positive finding of an increased amount of tyrosine in the urine. If the tyrosine concentration is higher than 500 mg per liter of urine, ie result No. 6, No. 7, No. 8, this is a positive result, a high content of tyrosine in the urine may indicate a more serious disease. In case of positive results, it is recommended to perform a more thorough examination by a general practitioner in order to exclude the risk of a possible serious illness.

5, Objective of the functional evaluation of in vitro diagnostic device for self-testing

This test describes the research method of using high performance liquid chromatography to detect the concentration of tyrosine in urine. The purpose was to proof if urinary tyrosine concentration of cancer patients is high.

6, Plan of functional evaluation of in vitro diagnostic device for self-testing

6a, Responsible person of functional evaluation of in vitro diagnostic device for self-testing

Laboratory skilled person is appointed as the person responsible for conducting the in vitro diagnostic device function evaluation.

The responsible persons who performed the laboratory samples, performed the tests and evaluated the results are fully qualified to work in the laboratory. Laboratories are responsible for performing the required experimental tests and have the necessary certification. The laboratories used to perform the tests are part of large hospitals, which are always listed in the individual test reports.

6b, List of laboratories used for functional evaluation of in vitro diagnostic device for self-testing

Special biological laboratory by state hospital

6c, Timetable for functional evaluation of in vitro diagnostic device for self-testing

1.9-10.9.2021

6d, Number of samples taken and tests performed for functional evaluation of in vitro diagnostic device for self-testing

110 samples of urine (Age of people: 18-60)

6e, Instructions for use, including a description of the conditions of use for functional evaluation of in vitro diagnostic device for self-testing

As part of the tests used in this evaluation of the function of the in vitro diagnostic device for self-testing, the laboratories were provided with standard instructions for use. In these tests, only urine collection was performed by the patients themselves (lay people), then the collected urine was immediately taken over by the laboratory to perform the test, the test itself and its evaluation was already performed by professional laboratory staff.

6f, Data on the function of the in vitro diagnostic device for self-testing

All function data are described in the chapters above and in particular in chapter "5, Objective of the evaluation of the function of the in vitro diagnostic device for self-testing".

6g, Documentation of functional evaluation of in vitro diagnostic device for self-testing

All documentation used is part of the technical documentation of the in vitro diagnostic device CarciReagent and is in the possession of the manufacturer (CNEU MEDICAL s.r.o.).

7, Basic information on the intention to functional evaluation of in vitro diagnostic device for self-testing

The manufacturer provided full information on the purpose of the in vitro diagnostic device, its detection principle, its proposed use, the method of evaluation of the test result and the interpretation of the result. Information was provided to those who collected the urine, performed the test, and evaluated the result and fully understood the function and use of the CarciReagent in vitro diagnostic device.

8, Design of experimental setup of functional evaluation of in vitro diagnostic device for self-testing

The procedure for validation tests is as follows:

1, Selection of tested persons according to the intention of individual evaluations (with regard to health status, age)

2, Urine samples collection: A total of 110 fresh and clean urine samples were collected and divided into groups according to different health status.

3, Sample test: Urine does not require pretreatment, and the injection volume is 10 microliters.

The high-performance liquid chromatograph is Vasian-5060, equipped with uV-100 ultraviolet detector and data processor. The chromatographic column uses a reversed-phase chromatographic column, the ultraviolet detector uses a wavelength of 280 nm ultraviolet light, the mobile phase is 15 mM phosphate buffer, and the pH is 6.5. The flow rate is 1.0 mL/min, and the column temperature is 30 ℃.

4, Record the test data and calculate the corresponding tyrosine content.

5, Carrying out the evaluation of all performed tests with regard to the goal of the given function evaluation

6, Processing and passing on the conclusions of the evaluation of the function, including informing about unexpected results or other undesirable situations during the implementation of the experiment

Aspects of tests for validation of in vitro diagnostic device function evaluation for self-testing:

Aspects that could affect the results of the CarciReagent in vitro diagnostic device performance evaluation were taken into account during the individual experimental tests. Emphasis was placed on qualified selection of test subjects, proper urine collection, adherence to the test procedure and its evaluation. The stability of the test set is set at 3 years. The persons who performed the testing and evaluation were acquainted with the aim of the individual experimental tests.

9, Structure of functional evaluation of in vitro diagnostic device for self-testing

We used high performance liquid chromatography to measure 110 cases. Among them, there are 19 healthy people, 10 males and 9 females. The average age is 38 years old, and the average concentration of tyrosine in urine is 0.295-0.331 μg/10 μl (see Table 1 for details). There were 45 cancer patients, 39 cases (positive) with tyrosine concentration in urine exceeding 2.30 μg/10 μl, and the positive rate accounted for 86.7%. All 46 patients with non-cancer diseases were negative (see Table 2 for details). Among non-cancer patients, there are diseases such as tuberculosis, pneumonia, hepatitis, liver cirrhosis, and gastritis. Among various cancers, gastric cancer, liver cancer, and nasopharyngeal cancer have a high positive rate (see Table 3 for details).

In particular, it should be pointed out that this method can help differential diagnosis of clinically difficult cases of cancer. For example, in a male patient, the liver scan has an occupying lesion, and the fetal thyroid gland is higher than 400 nanograms. The clinicians all considered liver cancer, but this test was negative. After long-term observation, the level of fetal thyroid gland gradually decreased to normal, and finally rejected Diagnosis of liver cancer. Another female patient had abdominal pain for two months. There were no obvious lumps in clinical examination, but the test

was positive. After barium enema, colon lesions were found. The laparotomy was confirmed to be colon cancer. After resection, the test turned negative.

Table 1: The average concentration of tyrosine in the urine of healthy people

Sex	Number of cases	Age range	Average age	Urinary concentration (μg/10 μl)
Female	9	15-51	34	0.217-0.324
Male	10	11-64	42	0.322-0.331
Total	19	11-64	38	0.295-0.331

Table 2: Analysis of tyrosine concentration in urine

Classification	Number of cases	UCC<2.30 μg/10 μl	UCC≥2.30 μg/10 μl
Cancer patient	45	6	39
Non-cancer patient	46	46	0
Healthy person	19	19	0

Table 3: Measurement results of cancer patients

Cancer patient	Number of cases	UCC≥2.30 μg/10 μl
Liver cancer	6	6
Stomach cancer	5	5
Nasopharyngeal carcinoma	3	3
Lung cancer	8	6
Esophageal cancer	4	3
Cervical cancer	4	3
Lymphoma	4	3
Other cancers	8	7
Total	45	39 (86.7%)

10, Records of tests of functional evaluation of in vitro diagnostic device for self-testing

All detailed documentation of the records of functional tests performed in the laboratory of hospital is available only to this institution. The procedures were performed in accordance with applicable laws and regulations. After performing the tests, the laboratory prepared summary and evaluation with data that are not subject to personal data protection. These have been received by the manufacturer and are part of the technical documentation for the in vitro diagnostic device.

11, Observations and unexpected results by functional evaluation of in vitro diagnostic device for self-testing

Unexpected results were not observed during the experimental tests, no deficiencies were reported with the test kits, and the tests did not need to be repeated.

12, Evaluation of individual experimental tests and functional evaluation report of in vitro diagnostic device for self-testing

There is a significant difference between the two in the measurement of urine that has passed through the high-performance liquid chromatographic column. The urinary tyrosine concentration of cancer patients is very high, on the contrary, the content of this substance in the urine of healthy people is very low. This method is simple and feasible, with an accuracy rate of 86.7%.

13, Changes during the functional evaluation study or re-evaluation

No changes were made during the evaluation study or re-evaluation.

14, Protection and safety of the persons under investigation

The protection and safety of the persons under investigation were fully ensured and no person was exposed to any danger. The protection of the personal data of the persons who took part in the testing was fully ensured.

15, References and list of documents

L.R. Snyder, J.J. Kirkland, J.L. Glajch (Eds.), Practical HPLC Method Development, Wiley, New York (1997), pp. 439-478
https://www.gbv.de/dms/ilmenau/toc/215662946.PDF

Zhen Wei, Xiaoyi Liu, Chunming Cheng, Wei Yu and Ping Yi, Metabolism of Amino Acids in Cancer
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7835483/

Peng Gao, Xin Huang, Xue-Yan Fang, Hui Zheng, Shu-Ling Cai, Ai-Jun Sun, Liang Zhao, and Yong Zhang, Application of metabolomics in clinical and laboratory gastrointestinal oncology
Application of metabolomics in clinical and laboratory gastrointestinal oncology - PMC (nih.gov)