Carci Reagent

CNEU MEDICAL s.r.o.
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Application of Visible Spectrophotometry to Detect Monohydroxyphenols in Urine

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

No.: AVSD/ZYB/2021

Introduction

Select monohydric phenols (tyrosine) as the standard substance, a detection method for the determination of monohydroxyphenolic (MHP) in urine was established by visible spectrophotometry. We used total of 620 cases of urine from healthy people (18-60 years old). In addition, 153 patients diagnosed with cancer were sellected (including 35 cases of nasopharyngeal cancer, 35 cases of ovarian cancer and cervical cancer, 20 cases of colorectal cancer, 31 cases of liver cancer, and 32 cases of gastric cancer). This test is to detect whether the monohydroxyphenolic substance (MHP) level in the urine of cancer patients is significantly different from that of normal people by visible spectrophotometry.

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 is to detect whether the monohydroxyphenolic substance (MHP) level in the urine of cancer patients is significantly different from that of normal people by visible spectrophotometry.

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**
Mr. Hu Min (Quality Inspector) 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
Third Affiliated Hospital of Sun Yat-sen University

6c, Timetable for functional evaluation of in vitro diagnostic device for self-testing
10-25 June, 2021

6d, Number of samples taken and tests performed for functional evaluation of in vitro diagnostic device for self-testing
Total 773 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 tool for self-testing, the laboratories were provided with standard instructions for use (Chinese version). 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 773 fresh and clean urine samples were collected and divided into groups according to different kinds of sickness.
3, Sample test: Take a certain amount of tyrosine standard solution, add an appropriate amount of mercury nitrate, 90～92 ℃ water bath for 15 minutes, 3 200r/min centrifugation for 5 minutes, take the supernatant and add $NaNO_2$, control the total volume of the reaction system to 3ml, and let it stand for a certain period of time. Centrifuge at 200r/min for 5min and use a 1cm cuvette to determine the OD value. In order to find out the maximum absorption wavelength, the optimal dosage of mercury nitrate, the optimal dosage of the developer $NaNO_2$, acidity, color development

time, etc. Determine the optimal conditions of the system, establish a method for determining the content of monohydroxyphenols, and use this method to determine the OD value of urine MHP in various normal populations and cancer patients.
4, The established experimental method was used to determine the MHP content in urine samples of 620 normal people and 153 cancer patients. According to the calculation formula, the sensitivity, specificity, positive predictive value and negative predictive value of this experimental method were counted.
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.

9, Structure of functional evaluation of in vitro diagnostic device for self-testing
Within a certain concentration range, the tyrosine content and the absorbance showed a good linear relationship. When using this method to determine the urine monohydroxyphenols of various normal people and tumor patients, the sensitivity was 78.15% and the specificity was 96.0 %, the positive predictive value is 85.4%, and the negative predictive value is 93.4%.

Selection of experimental conditions

Maximum absorption wavelength
Using tyrosine as the standard, the reaction system was prepared according to the above experimental method, and the reaction system was scanned in the range of 400-600nm. The maximum absorption wavelength of the obtained absorption curve was at 500nm.
The results are shown in Table 1.

Table 1 Maximum absorption wavelength

Wave length (nm)	460	470	480	490	500	510	520
OD value	0,385	0,443	0,475	0,500	0,520	0,510	0,474

The best dosage of various reagents
On the basis of testing the dosage range of each reagent, orthogonal test is used to determine the optimal dosage of each reagent. The results showed that: in a 3ml reaction system, the optimal dosage of 2mol/L mercury nitrate was 0.3ml; 0.01mol/L $NaNO_2$ was 0.5ml; 9mol/L HNO_3 was 0.2ml.

Color development time
According to the above experimental method, after adding 0.01mol/L NaNO20.5ml for color development, the absorbance value decreases with the increase of the reaction time, and the change is small after 36 minutes. In this experiment, the color development time is selected as 36 minutes.

The results are shown in Table 2.

Table 2 Color development time

Time (min)	6	12	18	24	30	36	42	48	54
OD value	0,690	0,510	0,399	0,327	0,283	0,243	0,243	0,243	0,243

Linear range
Tyrosine shows a good linear relationship in the concentration range of 0.4～3.2mmol/L.
The results are shown in Table 3.

Table 3 Standard curve

Tyrosine concentration (mmol/L)	0,4	0,8	1,2	1,6	2	2,4	2,8	3,2
OD value	0,045	0,092	0,148	0,192	0,230	0,293	0,330	0,381

Sample recovery experiment
Take 7 urine samples of the same normal person, 6 of which are added with different amounts of tyrosine standard substance (0.01mol/L). According to the urine sample processing method, the urine sample without tyrosine is used as a blank. The absorbance (A) n=6 was measured at 500nm, and the average recovery was 98.1%, SD1.9%.

Determination of urine sample
Take 2mL of urine from normal people and cancer patients and measure the OD value at 500nm according to the above-mentioned test method for the determined dosage of reagents.
It can be seen from the experimental results that the OD value of MHP in normal urine samples is 0.11±0.089. If the content of tyrosine is used to reflect the content of MHP in urine, the range of MHP content in normal urine samples is 0.18∽1.64mmol/L (about It is 32.76∽298.5 mg/L), and the median content is 0.91mmol/L (about 165.6mg/L). (The molecular weight of tyrosine is 182) It can be seen from the experimental results that the OD values of MHP in the urine samples of cancer patients are higher than those of normal people ($P < 0.05$). Among them: the OD value of MHP in the urine samples of nasopharyngeal cancer patients is 0.54±0.18, and the positive rate is 81.4%. If the content of tyrosine is used to reflect the content of MHP in the urine, the MHP in the urine samples of nasopharyngeal cancer patients The content range is 2.95∽5.90mmol/L (approximately 536.9∽1073.8mg/L), and the median content is 4.43mmol/L (approximately 806.3mg/L). The OD value of MHP in urine samples of patients with ovarian cancer and cervical cancer is 0.63±0.045, and the positive rate is 83.3%. If the content of tyrosine is used to reflect the content of MHP in the urine, the level of MHP in the urine samples of patients with ovarian cancer and cervical cancer is the

content range is 4.80∽5.54mmol/L (approximately 873.6∽1008.3mg/L), and the median content is 5.17mmol/L (approximately 940.9mg/L).
The OD value of MHP in urine samples of liver cancer patients is 0.37±0.14, and the positive rate is 70.0%. If the content of tyrosine is used to reflect the content of MHP in urine, the range of MHP content in urine samples of liver cancer patients is 2.0∽4.18mmol/ L (about 364∽760.8mg/L), with a median content of 3.09mmol/L (about 562.4mg/L).
The OD value of MHP in urine samples of gastric cancer patients is 0.51±0.089, and the positive rate is 71.5%. If the content of tyrosine is used to reflect the content of MHP in urine, the range of MHP content in urine samples of gastric cancer patients is 3.45∽4.91mmol/ L (approximately 627.9∽893.6mg/L), the median content is 4.18mmol/L (approximately 760.8mg/L).
After calculation, the sensitivity of this method is 78.1%, the specificity is 96.0%, the positive predictive value is 85.4%, and the negative predictive value is 93.4%.

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
Visible spectrophotometry to detect the content of monohydroxyphenols in urine has practical value and can be used as an indicator for quantitative tumor detection. The test results of this method show that the level of MHP in the urine of cancer patients is significantly different from that of normal people.
From the experimental process, this method has convenient sampling, avoids the pain of blood drawing and the chance of cross-infection, is simple and quick to operate, does not require special equipment, and is low in cost. Therefore, it is suitable for the promotion of general tumor surveys in primary hospitals and remote mountainous areas.

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

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