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Comparison test of CarciReagent and high-performance liquid chromatograph (HPLC) test results

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

No.: CTU-HPLC/ZYB/2021

Introduction

In this test, we want to compare the CarciReagent test kit with HPLC to detect the tyrosine content and his approximate amount in urine. In total of 875 samples were tested. Our purpose was to test whether there is a significant difference between results by CarciReagent and HPLC for the detection of tyrosine in urine.

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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

In this test, we want to compare the CarciReagent test kit with HPLC to detect the tyrosine content and his approximate amount in urine. In total of 875 samples were tested. Our purpose was to test

whether there is a significant difference between results by CarciReagent and HPLC for the detection of tyrosine in urine.

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**

Qualified laboratory staff is appointed as the team 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

Qualified laboratory by State Hospital

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

10-20 September, 2021

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

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

For testing we used these batches LOT 20190303, LOT 20190501, LOT 20190702 (all valid for three years), we prepare 330 testing set of each batches.

Because three batches were used, and the aim was not to compare batches with each other, the test procedures were performed randomly, there were 300 tests from batch 20190303, 300 tests from batch 20190501, and 275 tests from batch 20190702.

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 875 fresh and clean urine samples were collected and performed by CarciReagent and HPLC tests, respectively.

3, Sample test:

CarciReagent test:

Suck 3ml of the urine sample with a urine pipette, add it into the ampoule bottle containing the reagent, and let it stand. After the reaction time is reached, observe the color of the precipitate and compare it with the standard color chart, and finally record the accurate color result. The judgment criteria are:

a, Negative: The reaction result is colorless/pale yellow/reddish.

b, Positive: The reaction result is light red/pink/rose red/brown red/dark rust red.

HPLC 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 for CarciReagent and HPLC tests, respectively.

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

Test results: Reagent detection reaction color	Corresponding urine tyrosine content detected by the reagent	Number of inspections	Corresponding urine tyrosine content detected by HPLC	Maximum deviation rate between reagent detection and HPLC detection
Light red	250±30mg/L	70	1.95-2.37μg/10μL (=195-237mg/L)	11.4%
Pink	333±60mg/L	158	2.52-3.25μg/10μL (=252-325mg/L)	7.7%
Rose red	500±150mg/L	367	3.37-6.38μg/10μL (=337-608mg/L)	3.7%
Brown-red	1000±350mg/L	245	6.51-11.79μg/10μL (=651-1179mg/L)	0%
Deep rust red	2000±700mg/L	35	14.36-17.83μg/10μL (=1436-1783mg/L)	0%
Total number of inspections		875	Maximum deviation rate of the two methods	11.4%

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

We used CarciReagent and high-performance liquid chromatography (HPLC) to detect the tyrosine content in the urine of 875 cancer patients (the source of urine was morning fresh urine). After testing, the results of the CarciReagent and the high-performance liquid chromatograph (HPLC) are consistent.

The maximum deviation rate of the two detection methods is 11.4%, and the deviation mainly occurs in the areas where the reagent reaction color is light red and pink, that is, the relatively light color area where the reagent reaction result is positive. The reasons for the deviation rate are specificity and sensitivity affected by the color of urine itself and affected by interference factors, especially tyrosinase inhibitor drugs taken by tumor patients.

Through the comparison test, in the rose red and brown red areas where the reaction color ratio of the tumor patient reagent is higher, the deviation rate of the detection results of the two methods is extremely low, which proves that the detection result of the CarciReagent has a high stability and accuracy.

Analysis shows high correlation between results of the CarciReagent and high-performance liquid chromatograph (HPLC).

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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