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Evaluation of the function of the in vitro diagnostic device for self-testing CarciReagent

(According to the standard EN 13612:2002)

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Introduction

The aim of this evaluation study of an in vitro diagnostic device for self-testing marketed in the EU under the name CarciReagent is to evaluate its purpose in a broader perspective. Thus, this evaluation will include individual evaluations, literature searches and other tests performed (list in the annex), which confirm the functional purpose of the device and further supplement the necessary information necessary for placing on the market. The standard EN 13612:2002 was used for this purpose.

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

The aim is to confirm the functionality of the test kit for detecting the approximate amount of monohydroxyphenol metabolites (tyrosine) in the patient's urine. The tester should therefore be able to find monohydroxyphenol metabolites (tyrosine) in the urine and detect it in the form of coloration (Chemical chromogenic method). The test result is then compared with a color scale, which determines a negative or positive result. Since the test is semi-qualitative, the color scale will determine both the negativity or positivity of the result, as well as the approximate amount of monohydroxyphenol metabolites (tyrosine) in the urine according to the attached table.

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

Second Affiliated Hospital of Nanchang University,
Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine,
Second Affiliated Hospital of Nanjing Medical University
Shanghai Tenth People's Hospital,
Shanghai Changhai Hospital,
Zhongshan Hospital Affiliated to Fudan University,
Guangdong Provincial People's Hospital,
Third Affiliated Hospital of Sun Yat-sen University

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

Experimental tests and literature searches were performed from 2016 to 2021. The time schedule for each phase is given in the section Structure of functional evaluation of in vitro diagnostic device for self-testing.

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

The total number of urine samples taken, and tests performed is based on individual experimental tests. The total number of people who participated in the testing is 10,048 (real number of tests). These were men and women aged 18-60. For testing purposes, the manufacturer planned to provide a total of 12,000 tests (Test LOT 20190305 total 4,000 pieces, Test LOT 20190515 total 4,000 pieces, Test LOT 20190722 total 4,000 pieces. For stability testing (see Stability testing 2016 and 2017), which was performed in the production laboratory, the factory provided for production laboratory a sufficient number of test kits of the required batches according to the requirements of the team leader of testing procedure.

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 (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, Collecting all experiment tests, literature searches and other tests performed with regard to the goal of the given function evaluation
2, Carrying out analysis of all particular individual evaluations (experiment tests, literature searches and other tests performed) with regard to the goal of the given function evaluation
3, Processing and passing on the conclusions of the evaluation of the function, including informing about unexpected results or other undesirable situations during the functional evaluation.
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

The functional evaluation of in vitro diagnostic device is divided into 11 phases, namely:

1, Stability testing according to EN ISO 23640: 2015
a, Test period October 2016- December 2020 for tested batches 20161003, 20161030, 20161108
- document 6_5 Stability testing 2016_052022
b, Test period March 2017-August 2021 for tested batches 20170303, 20170501, 20170702
- document 6_6 Stability testing 2017_052022
Objective: Stability study purpose
In order to investigate the stability of the reagents, three batches of products were subjected to the Validity period stability verification test (Accelerated testing), Stability of validity period test (Real time testing), Transport stability test, Stability test after expiry date. The validity period and transportation conditions of the product are determined according to the test results.
Conclusion
All test according to EN 23640:2015 was successfully done. The product has required quality level and performance.

2, Testing the reaction time of the test result depending on the temperature according to EN ISO 23640: 2015
Test period 3.3.-6.3.2017 for tested batches 20170301, 20170302, 20170303
- document 6_9, Result reaction based on temperature and time CarciReagent_052022
Objective: According to the results of the study on the high and low temperature stability of the urine monohydroxyphenol metabolite detection reagent to test the influence of the reaction time under different temperature conditions on the reagent.
Conclusion: There will be no influence on the test results under the test environment at 5°C to 40°C. It is best to make a judgment on the result within 10 minutes when using this detection reagent for detection. Avoid too long to judge the result inaccurately.

3, Literary research - Diseases associated with abnormal tyrosine metabolism according to EN 13612:2002
Test period 1.2.-15.4.2021
- document 6_1d, Diseases associated with abnormal tyrosine metabolism_052022
Objective
Carry out a literature search in accessible scientific publications and summarize the abnormal tyrosine metabolism related diseases which probably affect the results of CarciReagent test.
Conclusion
According to the above literature review, diseases that can cause low levels of tyrosine in urine include Parkinson's disease, depression (tyrosine deficiency), Albinism (genetic disorder), Phenylketonuria (PKU), Tyrosinemia (metabolic hereditary disorders). Diseases that can cause high levels of tyrosine in urine are malignant tumors, mainly include digestive tract malignant tumors (gastric cancer, bowel cancer), liver cancer, nasopharyngeal cancer, lymphoma, breast cancer, gynecological malignant tumors, lung cancer, etc. Diseases that probably increase tyrosine in the urine are pigment disorders (freckles, brown spots), diabetes, stomach ulcers and gastritis.

4, Literary research "Tyrosine as a significant marker in urine for different cancer" according to EN 13612:2002

Test period 1.5.-30.6.2021

- document 6_4e, Tyrosine as significant marker in urine for different cancer_052022

Objective

Summarize the literatures about the abnormal tyrosine metabolism plays an important role in tumor diseases. Lay the foundation for the possible using of the CarciReagent test.

Conclusion

It is shown that the content of tyrosine in urine is an important marker of tumor diseases, which can be used for the screening of certain tumor diseases.

5, Experimental test "Application of Visible Spectrophotometry to Detect level of Tyrosine in Urine" according to EN 13612:2002

Test period 10.-25.6.2021

Number of tests: 773 tests (620 in healthy and 153 in patients with various types of cancer

- document 6_4d, Application of Visible Spectrophotometry to Detect level of Tyrosine in Urine_052022

Objective

The 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.

Conclusion

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 monohydroxyphenolic (MHP) in the urine of cancer patients is significantly different from that of normal people.

6, Experimental test „Summary of clinical trials of CarciReagent in China (summer 2021) according to EN 13612:2002

Test period 1.6.-30.9.2021

Number of tests: 8,078 people (4,375 people with various types of cancer, 3,703 people without cancer, see the description in the document)

Batch tested: 20190305, 20190515, 20190722

- document 6_4b, Summary of clinical trials of CarciReagent in China (summer 2021) _052022

Objective

In this test, we want to proof if this medical device can help with his result of indicative volume of tyrosine in human urine for detection of possible malignant tumor diseases. Looking for particular kind of cancer was not objective of this test. Our purpose was to set relation between level of monohydroxyphenol metabolites (tyrosine) and malignant/nonmalignant diseases.

Conclusion

After testing, the malignant tumor group (patients diagnosed with cancer by the hospital) had an average positive rate of 96.7%, and the non-malignant tumor group (diagnosed non-cancer patients, including: patients with tyrosine-related diseases, patients with common diseases, and normal health people) had an average positive rate of 3.35%. And by only normal health people was average positive rate by 0,6%. The positive rate of the malignant tumor group was significantly higher than that of the non-malignant tumor group. The difference between the two detection results was statistically significant.

Malignant tumors are the most important cause of positive test results, and they are statistically highly probable.
In the non-malignant tumor group, the positive rate of patients with pigment disorders (chloasma), gastritis, gastric ulcer and diabetes is relatively high, which is one of the causes that may lead to a positive test result. However, the overall level of the positive proportion is low, which is a statistically small probability situation.
In the non-malignant tumor group, the positive rate of other types of common diseases and people in normal health status is very low. The analysis may be mainly due to the false positive results caused by the stress response and interference factors of the human immune system, and there is no statistics significance.
After a large number of clinical tests of this reagent, the positive detection rate of malignant tumors is significant. According to the statistical analysis, the positive results have a great probability to detect malignant tumor diseases. The positive results of other diseases are very low, or none.

7, Experimental test "Sensitivity and specificity test report of CarciReagent" according to EN 13612:2002
Test period 1.6.-30.9.2021
Number of tests: 8,078 people (4,375 people with various types of cancer, 3,703 people without cancer, see the description in the document)
Batch tested: 20190305, 20190515, 20190722
- document 6_1g, Sensitivity and specificity test report of CarciReagent_052022

Objective
In this test, we want to verify the sensitivity and specificity of the medical device. Our purpose was to set up the stability and specificity of the medical device in practical applications.
Conclusion
Through a large number of clinical detection test studies, the detection sensitivity for the cancer patient group (hospital-diagnosed cancer patients) was 96.7%, and the detection sensitivity for other diseases was lower. The specificity of the detection of this reagent is 99.4%. This medical device is only used for indicative detection. By detecting the abnormal metabolism of monohydroxyphenol in human body, the probability of tumor disease can be warned. The tested person must further investigate and diagnose through other detection methods of professional medical institutions.

8, Food Interference Factor Test according to EN 13612:2002
Test period 1.-15.9.2021
Number of tests: 212 people (repeated tests according to plan)
Batch tested: 20190305, 20190515, 20190722
- document 6_1f, Food Interference Factor Test Report_052022
Objective
To study whether the consumption of foods that increase tyrosine levels in the body or foods rich in pigments within 48 hours before testing with medical device can lead to false-positive results.
Conclusion
Through 3 consecutive tests, it has been proved that within 48 hours before using the reagent test, eating foods that can increase the content of tyrosine in the body may interfere with the test results, resulting in false positive test results. Eating foods rich in pigment may excrete red urine, making the urine sample invalid and not suitable for continued testing. However, after 48 hours of eating these foods, the reagent test was carried out, and the interference factor basically did not exist.

9, Literary research "Analysis of possible interference factors in urine tyrosine detection reagent" according to EN 13612:2002

Test period 1.-30.9.2021
- document 6_1a, Analysis of possible interference factors in reagent_052022

Objective

Aims of this research paper is to carry out a literature search in accessible scientific publications and find out the factors of possible affecting the level of monohydroxyphenol metabolites in the patient's urine in order to eliminate false negative or false positive results of CarciReagent test.

Conclusion

According to the above literature review, factors that may cause tyrosine content changes in urine are: food or drugs that can decompose and activate a large amount of amino acids; certain drugs that inhibit the breakdown of amino acids; patients with gastritis and gastric ulcers who are accompanied by Helicobacter pylori infection, as well as patients with diabetes; people's physical condition. Factors that can cause changes in urine color include: ed urine, dark yellow urine, blue urine, and black urine.

10, Experimental test "Detecting the concentration of tyrosine in urine with HPLC to diagnose cancer" according to EN 13612:2002

Test period 1.-10.9.2021
Number of tests: 110 people
Test equipment: HPLC chromatograph Vasian-5060 with uV-100 ultraviolet detector and data processor (Second Affiliated Hospital of Nanchang University)
- document 6_4a, Detecting the concentration of tyrosine in urine with HPLC to diagnose cancer_052022

Objective

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.

Conclusion

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%.

11, Experimental test "Comparison test of CarciReagent and HPLC test results" according to EN 13612:2002

Test period 10.-20.9.2021
Number of tests: 875 people with various types of cancer
Batch tested: 20190305, 20190515, 20190722
Test equipment: HPLC chromatograph Vasian-5060 with uV-100 ultraviolet detector and data processor (Second Affiliated Hospital of Nanchang University)
- document 6_4c, Comparison test of CarciReagent and HPLC test results_052022

Objective

In this test, we want to compare the CarciReagent 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.
Conclusion
After testing, the results of the reagent 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).
Based on the evaluation of the above documents, an evaluation report will be compiled containing descriptions of the individual phases, analysis of the results and a conclusion on the declared function of the in vitro diagnostic device for self-testing “CarciReagent”.

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 laboratories of various hospitals is available only to these institutions. The procedures were performed in accordance with applicable laws and regulations. After performing the tests, the laboratories prepared summaries and evaluations 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

The test kit (CarciReagent) is for detecting the approximate amount of monohydroxyphenol metabolites (tyrosine) in the patient's urine. The basic principle of the test is based on the improved method of 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 fresh morning urine (middle urine stream), the reaction color cascade can be used to determine if urine samples contain increased amounts of these metabolites.
Through literature research and experimental tests (Application of Visible Spectrophotometry to Detect level of Tyrosine in Urine and Detecting the concentration of tyrosine in urine with HPLC), diseases that can cause high levels of tyrosine in urine are malignant tumors, mainly include digestive tract malignant tumors (gastric cancer, bowel cancer), liver cancer, nasopharyngeal cancer,

lymphoma, breast cancer, gynecological malignant tumors, lung cancer, etc. The level of monohydroxyphenolic (MHP) in the urine of cancer patients is significantly higher than that of normal people. Diseases that probably increase tyrosine in the urine are pigment disorders (freckles, brown spots), diabeteses, stomach ulcers and gastritis.
The quality performance of the product was determined by studying the stability of the reagents. By studying the reaction time and temperature, the reaction temperature and time were determined: test environment at 5°C to 40°C and make a judgment on the result within 10 minutes (recommendation is for 3-5 minutes).

Factors that cause changes in tyrosine content in urine and urine color will affect the test results. Factors that may cause tyrosine content changes in urine are food or drugs that can decompose and activate a large amount of amino acids; certain drugs that inhibit the breakdown of amino acids; patients with gastritis and gastric ulcers who are accompanied by Helicobacter pylori infection, as well as patients with diabetes; people's physical condition. Factors that can cause changes in urine color include red urine, dark yellow urine, blue urine, and black urine. Furthermore, eating foods that can increase the content of tyrosine in the body may interfere with the test results, resulting in false positive test results.

The sensitivity and specificity of this reagent have been done in a large number of clinical trials in China. The results show that malignant tumor group (patients diagnosed with cancer by the hospital) had an average positive rate of 96.7%. The non-malignant tumor group (diagnosed non-cancer patients, including patients with tyrosine-related diseases, patients with common diseases, and normal health people), had a positive rate of only 3.35%. The positive rate of the malignant tumor group was significantly higher than that of the non-malignant tumor group. The difference between the two detection results was statistically significant.
In summary, the test kit for detecting the approximate amount of monohydroxyphenol metabolites (tyrosine) in the patient's urine can be used as diagnostic device in vitro for self-testing. The tester is therefore able to find monohydroxyphenol metabolites (tyrosine) in the urine and detect it in the form of coloration (Chemical chromogenic method). The test result is then compared with a color scale, which determines a negative or positive result. Since the test is semi-qualitative, the color scale will determine both the negativity or positivity of the result, as well as the approximate amount of monohydroxyphenol metabolites (tyrosine) in the urine according to the attached table.

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

References have been made in each particular individual evaluations (experiment tests, literature searches and other tests performed). These references are available in technical documentation.