CNEU MEDICAL s.r.o.
Jeřábkova 1459/8, 149 00 Praha-Chodov, Czech Republic

Sensitivity and specificity test report of CarciReagent

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

No.: SSTR/ZYB/2021

Introduction

In this test, we want to verify the sensitivity and specificity of the medical device. We used total of 8.078 urine samples (include cancer patient group, tyrosine-related diseases patient, common diseases patient, and normal people). All samples were divided into 3 groups for testing. Our purpose was to set up the stability and specificity of the medical device in practical applications.

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
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. For determination of sensitivity and specificity of medical device will be use data received by other experimental test (Summary of clinical trials of CarciReagent tester kit inChina; SCT/ZYB/2021).

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

6c, Timetable for functional evaluation of in vitro diagnostic device for self-testing
1.6-30.9.2021

6d, Number of samples taken and tests performed for functional evaluation of in vitro diagnostic device for self-testing
8.078 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). Because three batches were used, we made three groups, there were Group 1 - 2700 tests from batch 20190303, Group 2 - 2700 tests from batch 20190501, and Group 3 - 2700 tests from batch 20190702. From Group 3 were 22 tests from batch 20190702 not used in this experiment.

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

2, Urine samples collection: A total of 8,078 fresh and clean urine samples were collected and divided into groups according to different health status.
3, Sample test: Suck 3ml of the urine sample with a urine pipette, add it into the ampoule bottle containing the reagent, and let it stand.
4, 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.
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
Cancer patients (mainly including: gastrointestinal malignant tumors, liver cancer, nasopharyngeal cancer, malignant lymphoma, breast cancer, gynecological malignant tumors, lung cancer, etc.).
Patients with tyrosine-related diseases (mainly including: pigmentary disorders (chloasma), Parkinson's disease, depression, albinism, urinary black acid urine and phenylketonuria, etc.).
Patients with common diseases (mainly including: diabetes, gastritis, gastric ulcer, esophagitis, enteritis, prostatic hyperplasia, tuberculosis, cholecystitis, viral hepatitis, lung infection, etc.).
Normal population (the normal population who is healthy and in good condition without any obvious pathological manifestations is screened by physical examination).

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Control group 1:				Batch 20190303		
Disease		Case	Result		Sensitivity	Specificity
			Positive	Negative		
Tested group 1	Cancer patient group	1459	1410	49	96,64%	
Tested group 2	Pigmentary disorders(chloasma)	24	5	19	20,83%	
	Diabetes	32	8	24	25,00%	
	Gastritis	56	9	47	16,07%	
	Gastric ulcer	27	4	23	14,81%	
	Parkinson's disease	14	0	14	0,00%	
	Depression	13	0	13	0,00%	
	Albinism	8	0	8	0,00%	
	Black aciduria and Phenylketonuria	4	0	4	0,00%	
	Esophagitis	26	2	24	7,69%	
	Enteritis	23	2	21	8,70%	
	Benign prostatic hyperplasia	10	1	9	10,00%	
	Tuberculosis	20	3	17	15,00%	
	Cholecystitis	39	4	35	10,26%	
	Viral hepatitis	35	4	31	11,43%	
	Lung infection	30	3	27	10,00%	
	Normal health population	880	5	875	0,57%	99,43%

Control group 2:				Batch 20190501		
Disease		Case	Result		Sensitivity	Specificity
			Positive	Negative		
Tested group 1	Cancer patient group	1458	1409	49	96,64%	
Tested group 2	Pigmentary disorders(chloasma)	22	5	17	22,73%	
	Diabetes	30	6	24	20,00%	
	Gastritis	54	7	47	12,96%	
	Gastric ulcer	25	3	22	12,00%	
	Parkinson's disease	12	0	12	0,00%	
	Depression	11	0	11	0,00%	
	Albinism	6	0	6	0,00%	
	Black aciduria and Phenylketonuria	3	0	3	0,00%	
	Esophagitis	24	1	23	4,17%	
	Enteritis	21	0	21	0,00%	
	Benign prostatic hyperplasia	8	0	8	0,00%	
	Tuberculosis	18	1	17	5,56%	
	Cholecystitis	37	2	35	5,41%	
	Viral hepatitis	33	2	31	6,06%	
	Lung infection	28	1	27	3,57%	
	Normal health population	910	4	906	0,44%	99,56%

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Control group 3:						Batch 20190702
Disease		Case	Result		Sensitivity	Specificity
			Positive	Negative		
Tested group 1	Cancer patient group	1458	1411	47	**96,78%**	
Tested group 2	Pigmentary disorders(chloasma)	22	5	17	22,73%	
	Diabetes	31	6	25	19,35%	
	Gastritis	56	9	47	16,07%	
	Gastric ulcer	26	4	22	15,38%	
	Parkinson's disease	14	0	14	0,00%	
	Depression	12	0	12	0,00%	
	Albinism	8	0	8	0,00%	
	Black aciduria and Phenylketonuria	3	0	3	0,00%	
	Esophagitis	24	1	23	4,17%	
	Enteritis	22	1	21	4,55%	
	Benign prostatic hyperplasia	10	1	9	10,00%	
	Tuberculosis	18	1	17	5,56%	
	Cholecystitis	39	2	37	5,13%	
	Viral hepatitis	34	3	31	8,82%	
	Lung infection	29	2	27	6,90%	
	Normal health population	872	7	865	0,80%	**99,20%**

Summary of results

Summary of results (3 control groups)						
Disease		Case	Result		Sensitivity	Specificity
			Positive	Negative		
Tested group 1	Cancer patient group	**4375**	**4230**	**145**	**96,69%**	
Tested group 2	Pigmentary disorders(chloasma)	68	15	53	22,06%	
	Diabetes	93	20	73	21,51%	
	Gastritis	166	25	141	15,06%	
	Gastric ulcer	78	11	67	14,10%	
	Parkinson's disease	40	0	40	0,00%	
	Depression	36	0	36	0,00%	
	Albinism	22	0	22	0,00%	
	Black aciduria and Phenylketonuria	10	0	10	0,00%	
	Esophagitis	74	4	70	5,41%	
	Enteritis	66	3	63	4,55%	
	Benign prostatic hyperplasia	28	2	26	7,14%	
	Tuberculosis	56	5	51	8,93%	
	Cholecystitis	115	8	107	6,96%	
	Viral hepatitis	102	9	93	8,82%	
	Lung infection	87	6	81	6,90%	
	Normal health population	2662	16	2646	0,60%	**99,40%**

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

Through a large number of clinical detection test studies, the detection sensitivity for the cancer patient group (hospital-diagnosed cancer patients) was 96.69%, 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.

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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