

# Performance evaluation of CarciReagent

No.: PEC/ZYB/2022

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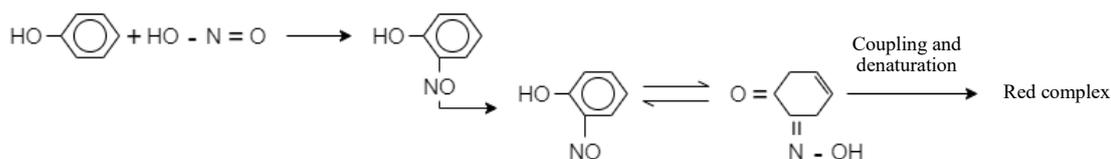
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## 1. Product introduction

Urine monohydroxyphenol is the general term for a group of amino acids and their various derivatives and metabolites. When there are cancer cells in the human body, the abnormal proliferation of cells triggers a stress response, which leads to abnormally increased metabolism of specific amino acids in the body, and the content of monohydroxyphenols (tyrosine) in the urine far exceeds that of normal people. This reagent can specifically react with the monohydroxyphenols (tyrosine) in the urine to determine the active level of cancer cells in the test subject. As a cancer early detection/screening reagent, it has the advantages of convenient use, simple operation, rapid detection, low cost, high accuracy, and strong specificity. It will become a clinical auxiliary detection method suitable for popularization.

## 2. Product Principle

This reagent can react specifically with the phenol nucleus of monohydroxyphenol, which originated from Millon's Test. It takes aromatic polar  $\alpha$ -amino acid containing phenolic hydroxyl group as the reaction substrate and uses the ortho position of phenolic hydroxyl group to easily react chemically with diazobenzene sulfonic acid. The orange-red substance is coupled, and the red shift of the protein and amino acid during the denaturation process is superimposed. The level of monohydroxyphenol metabolites in the urine of the subject is judged by color comparison with the standard color scale. The chemical reaction formula of the reaction principle (represented by the phenol nucleus of monohydroxyphenol) is as follows:



Judgment standard (compared with color code)

Negative: The result of the reaction is colorless/light yellow/reddish, marked as (-)

Positive: If the reaction result is light red/pink rose red/brown red/dark rust red, it is recorded as (+)

### 3. Product function

This reagent detection method is mainly to detect the content of monohydroxyphenol (tyrosine) in metabolites (urine) of the general normal population. Whether the test result is positive, it reflects whether there is abnormal metabolism of monohydroxyphenol (tyrosine) in the human body.

Through a large number of scientific research, clinical trial data and statistical probability analysis, people with positive test results of this reagent have a higher probability of active tumor cells in the body, so it can provide early warning reminders of tumor diseases for people with positive results. And it is recommended to go to a professional medical institution for further testing and diagnosis to achieve the purpose of early detection and early treatment.

This reagent is only a broad-spectrum detection. It can give early warning of the occurrence probability of tumor diseases by reflecting whether there is abnormal metabolism of monohydroxyphenol (tyrosine) in human body. It is not specific to a specific tumor cell and disease, cannot diagnose any disease type including malignant tumor, and cannot judge the period and stage of tumor diseases, The tester must further investigate and diagnose through other testing methods of professional medical institutions.

### 4. Evaluation plan

A total of 8078 urine samples were tested, of which:

1. Malignant tumor group (patients diagnosed with cancer by the hospital, mainly including: gastrointestinal malignant tumor, liver cancer, nasopharyngeal cancer, malignant lymphoma, breast cancer, gynecological malignant tumor, lung cancer, etc.), a total of 4375 samples were tested, of which 4230 were positive and 145 were negative, with an average positive rate of 96.7%.

2. In the non-cancer group (diagnosed non-cancer patients, including: patients with tyrosine-related diseases, patients with common diseases, and normal people), a total of 3,703 samples were tested, of which 124 were positive and 3,579 were negative. The average positive rate is 3.35%.

After a large number of clinical tests, the positive detection rate of malignant tumors is the highest. According to statistical analysis, the positive results of the detection have a great directional probability for malignant tumors, and the accuracy and sensitivity are high. While other diseases have a low degree of directivity and sensitivity.

## **5. Analysis of the reasons that may cause the test result to be positive**

### **5.1 Malignant tumor diseases:**

A large number of literature studies and clinical trials have proved that the abnormal increase in the metabolism of monohydroxyphenol (tyrosine) in the human body is the most likely cause of malignant tumor disease. In the large number of clinical trials that have been done in China, the malignant tumor group (patients diagnosed with cancer in the hospital) tested a total of 4375 samples, of which 4230 were positive and 145 were negative, with an average positive rate of 96.7%. The positive ratio of the malignant tumor group was significantly higher than that of the non-malignant tumor group, and the difference between the two detection results was statistically significant.

At the same time, research and clinical trials of a large number of documents have proved that: in different types of malignant tumors such as gastrointestinal malignancies (gastric cancer, bowel cancer), liver cancer, nasopharyngeal cancer, lymphoma, breast cancer, gynecological malignancies, lung cancer, etc. Has a high positive detection rate. Therefore, this reagent detection method can only provide an early warning reminder of whether the human body has a malignant tumor disease or the activity of tumor cells in the human body, and cannot clearly point to a specific type of malignant tumor disease.

The detection sensitivity for the cancer patient (gastric cancer, bowel cancer, liver cancer, nasopharyngeal cancer, lymphoma, breast cancer, gynecological malignancies, lung cancer, etc.) was 96.7%. The specificity of the detection of this reagent is 99.4%.

### **5.2 Some common diseases**

Research and clinical trials of literature have proved that the positive detection rate of patients with pigment disorders (chloasma), gastritis, gastric ulcer and diabetes is relatively high, generally between 5% - 25%, which may lead to detection. The result is one of the causes of positive. However, the overall level of positive proportions is low and does not have statistical significance. The detection sensitivity for other diseases (Pigmentary disorders, Diabetes, Gastritis, Gastric ulcer, Parkinson's disease, Depression, Albinism, black aciduria and phenylketonuria, Esophagitis, Enteritis, Benign prostatic hyperplasia, Tuberculosis, Cholecystitis, Viral hepatitis, Lung infection, etc.) was between 5% - 25%. The specificity of the detection of this reagent is 99.4%.

## **6. Applicable conditions for detection of this reagent**

The applicable object of this reagent detection is normal people without obvious disease manifestations and physical discomfort.

The tested person should eliminate all kinds of interference (don't drink alcohol, don't take all kinds of drugs, eat less high-protein, high-amino acid food and health products, and keep the body in a healthy environment and keep a good rest) within 48 hours before proceeding with the test.

People who have obvious disease symptoms and physical discomfort should seek medical treatment in time. If this reagent is used for testing, the body's overreaction and interference factors will cause the test results to be inaccurate.

An interfering experimental study to test whether the consumption of foods that increase tyrosine

levels in the body or foods rich in pigments within 48 hours before testing with CarciReagent can lead to false-positive results.

## 7. Monohydroxyphenol substances and tyrosine

Monohydroxyphenols in urine refer to the general name of a class of substances containing monohydroxyphenols in human urine, especially tyrosine, its derivatives and metabolites.

Literature research and clinical trials have proved that when tumor cells are active in human body, the content of tyrosine and its derivatives and metabolites increases most significantly, which is the main factor leading to the significant increase of monohydroxyphenol content in urine.

The composition of monohydroxyphenols is complex and diverse. In various medical experiments, tyrosine solutions are commonly used as standard solutions and reference solutions to quantitatively detect and calculate the content of monohydroxyphenols (tyrosine) in urine.

## 8. Testing of the accuracy of reagents results

### 8.1 Prepare a reference solution for testing the accuracy of reagents

The tyrosine standard solution is used as the reference solution for detecting the accuracy of the reagent.

8.1.1 Instruments and equipment: glass instruments, analytical balances, purified water machines

8.1.2 Reference solution preparation:

Positive reference solution preparation method: accurately weigh 2 g of tyrosine (analytical purity), fully dissolve it in 1L of purified water, prepare a tyrosine solution (original solution) with a concentration of 2 g/L, and then dilute it with purified water as required 2 times, 4 times, 6 times, 8 times, 10 times, 13.3 times the concentration of the series of reference solutions, their concentrations are 1000 mg/L, 500 mg/L, 333 mg/L, 250 mg/L, 200 mg/L, 150 mg/L. The negative standard solution is purified water.

8.1.3 Use a pipette to take 3 ml of measurement (reference solution of different concentrations) and drop it into the reagent bottle, shake it gently, compare and judge, and record the accurate color results. The judgment standard is compared with the color card:

Negative: the reaction result is colorless / light yellow / reddish, which is recorded as (-);

Positive: if the reaction result is light red / Pink / Rose Red / brown red / dark rust red, it is recorded as (+)

The critical value of negative and positive reagent test results is 250mg / L.

Number	1	2	3	4	5	6	7	8
Tyrosine solution dilution ratio	Purified water	1:13	1:10	1:8	1:6	1:4	1:2	stock solution
Tyrosine concentration (mg/L)	0	150	200	250	333	500	1000	2000
Marked value (Reaction color)	Colorless	light yellow	reddish	light red	pink	rose red	brown red	dark rust red
Positive/negative	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)

The OD value of monohydroxyphenols (MHP) in normal urine samples is  $0.11 \pm 0.089$ . 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 0.18-1.64 mmol/L (About 32.76-298.5 mg/L), the median content is 0.91 mmol/L (about 165.6 mg/L). (The molecular weight of tyrosine is 182)

The OD value of MHP in urine samples of patients with otorhinolaryngeal cancer is  $0.54 \pm 0.18$ , and the positive rate is 81.4%. If the content of tyrosine is used to reflect the content of MHP in urine, the range of MHP content in urine samples of patients with nasopharyngeal cancer It is 2.95-5.90 mmol/L (approximately 536.9-1073.8 mg/L), and the median content is 4.43 mmol/L (approximately 806.3 mg/L).

The OD value of MHP in urine samples of patients with ovarian cancer and cervical cancer is  $0.63 \pm 0.045$ , 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 The content range is 4.80-5.54 mmol/L (approximately 873.6-1008.3 mg/L), and the median content value is 5.17 mmol/L (approximately 940.9 mg/L).

The OD value of MHP in urine samples of liver cancer patients is  $0.37 \pm 0.14$ , 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 2.0-4.18 mmol/L (about 364-760.8 mg/L), with a median content of 3.09 mmol/L (about 562.4 mg/L).

The OD value of MHP in urine samples of gastric cancer patients is  $0.51 \pm 0.089$ , 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 3.45-4.91 mmol/L (approximately 627.9-893.6 mg/L), the median content is 4.18 mmol/L (approximately 760.8 mg/L).

Conclusion: It is reasonable and effective to set the negative and positive cutoff value of reagent test results to a concentration of 250 mg/L; the OD value of MHP in urine samples of cancer patients is higher than that of normal people ( $P < 0.05$ ).

## **9. Stability Research**

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.

## **10. Product component composition and raw material:**

Carton, manual, color card, certificate: raw material is white cardboard

Reagent container (ampoules): the raw material is neutral borosilicate glass

Plastic support, plastic support cover: the raw material is PET plastic sheet

Straws, plastic caps: raw materials are PE polyethylene white plastic particles

## 11. Conclusion

This medical device CarciReagent is mainly to detect the content of monohydroxyphenol (tyrosine) in urine of the general health population. Whether the test result is positive, it reflects there is abnormal level of monohydroxyphenol (tyrosine) in the human body. It can give early warning of the occurrence probability of serious sicknesses which can include malignant diseases. This reagent is only a broad-spectrum detection. It is not specific to a specific tumor cell and disease, cannot diagnose any disease type including malignant tumor, and cannot judge the period and stage of tumor diseases. The patient with positive result must be further investigate and diagnose through other testing methods of professional medical institutions.

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