



Check4®-Myoglobin Urine test

Myoglobin immuno-chromatographic rapid Test code QBX020MGL

I- PRINCIPLE

Myoglobin is an intra cellular haem protein involved in the storage and transfer of oxygen to muscle tissues⁽¹⁾. Its presence outside of the muscle cells is an indicator of muscle damage by physical or chemical agents, or restriction of oxygen or energy supply to the muscle. When released in this way, myoglobinuria results. This is the presence of myoglobin in the urine, resulting in a brown colour when present at high concentration. Urine myoglobin can cause acute renal failure by precipitation in the renal tubules and by conversion to products toxic to the tubules. Rapid diagnosis and treatment is therefore needed if recovery is to follow. Although muscle injury leading to myoglobinuria can be caused in a number of ways (e.g. severe electric⁽²⁾ shock, drug overdose and excessive exercise) crush injury⁽³⁾ is a common cause.

The Check4®-Myoglobin test is a rapid qualitative test assay for the detection of human myoglobin, which can be used with diluted urine. The method employs a unique combination of monoclonal-dye conjugate and polyclonal solid phase antibodies to identify myoglobin in the test samples with a high degree of specificity. As the sample flows through the absorbent device, the labelled antibody-dye conjugate binds to the myoglobin forming an antibody antigen complex. This complex binds to the anti-myoglobin antibody in the positive reaction zone (B) and produces a pink-rose colour band when myoglobin concentration is higher than 100 ng/ml. In the absence of myoglobin, there is no line in the positive reaction zone. The reaction mixture continues flowing through the absorbent device past the positive reaction zone and control zone. Unbound conjugate binds to the reagents in the control zone (C) producing a pink-rose colour band, demonstrating that the reagents are functioning correctly.

II-TEST KIT COMPONENTS

Each kit contains everything needed to perform 20 tests except saline.

- 1) Check4®-Myoglobin unit : 20
- 2) Disposable Plastic pipette 20
- 3) Diluent in a dropper bottle 5 ml
- 4) Instruction leaflet

III- STORAGE AND STABILITY

- 1- All Check4®-Myoglobin Test kit components should be stored at room temperature (but are stable from 4° to 30°C).
- 2- Do not freeze the test kit.
3. The Check4®-Myoglobin Test kit is stable until the expiry date stated on the package label.

IV- PRECAUTIONS

- 1- For "IN VITRO" diagnostic use and professional use only.
- 2- Handle all specimens as if they contain infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1% solution of sodium hypochlorite for one hour before disposal.
- 3- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
- 4- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
- 5- Avoid any contact between hands and eyes or nose during specimen collection and testing.
6. Do not use beyond the expiration date stated on the package label.
7. Do not use a test from a damaged protective wrapper.

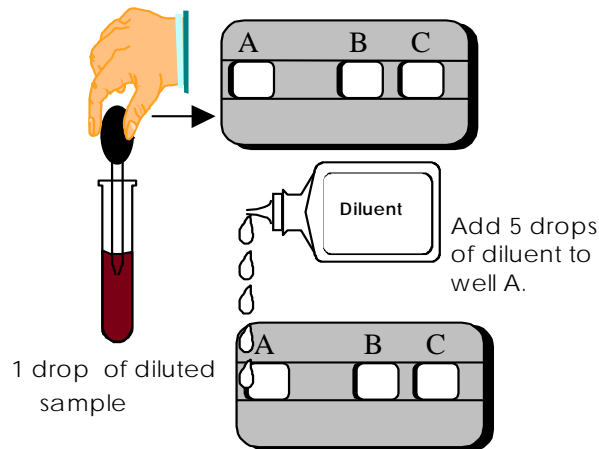
V- SPECIMEN COLLECTION AND PREPARATION

Urine

- 1- Collected specimens should be treated as if potentially infectious.
- 2.-Myoglobin in urine deteriorates within 100 minutes unless the sample is buffered (6.5-8.8) but testing should still take place within 24 hours⁽⁴⁾. Transport of samples should take place in 2% sodium bicarbonate.
- 3-It should also be noted that deterioration of urine myoglobin can be influenced by other urinary factors apart from pH⁽⁴⁾

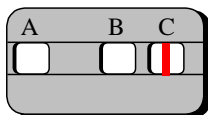
VI- ASSAY PROCEDURE

1. Allow samples and Check4®-Myoglobin test cassettes to reach room temperature prior to assay.
2. Remove the cassette from its protective wrapper and place on a flat surface.
3. Dilute the sample 1 + 5 with saline
4. Fill the dropper with sample and dispense 1 drop (20 µl) into sample well A
5. Add 5 drops (200 µl) of diluent into sample well A.
6. Read the results after 5 and up to 10 minutes.
7. All negatives should be further diluted 1+10 in saline and the test re-run

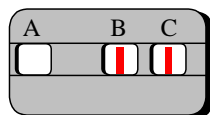


VII- READING TEST RESULTS

Interpretation of the results after 10 minutes.



Negative test



Positive test

Negative:

Only one coloured band appears in the control window (C). At this stage we recommend that the diluted sample is further diluted 1+10 in saline and the test rerun. If still negative but the urine is clearly brown then a range of increasing dilutions should be run i.e. 1+50, 1+100 and 1+1000. If positive then very high levels of myoglobin are present.

Positive:

In addition to the control band, a clearly distinguishable band also appears in the test window (B).

Inconclusive:

If there are no distinct colour bands in either the test or control windows, the test is inconclusive. It is recommended that in this case, the test should be repeated with a fresh sample.

VIII- PERFORMANCE CHARACTERISTICS

A) Sensitivity :

The analytical sensitivity of the test is 100 ng Myoglobin/ml. Concentrations below 100 ng/ml may also be detected occasionally.

B) Specificity : An evaluation of the Check4® Myoglobin kit for the detection of urine myoglobin was carried out at a major UK teaching hospital⁽⁵⁾ which receives a significant number of motor accident victims.

Initially the test sensitivity was a problem: The detection level of Check4® Myoglobin is 100ng myoglobin/ml. Since concentrations of 300ng to 2g myoglobin/ml may be found in myoglobinuria it was clear that prozoning could occur (i.e. the appearance of false negative results due to overloading of the capture antibody). To avoid this dilutions were made before testing with Check4® Myoglobin.

The evaluation was made with 64 urine samples taken from crush injury victims. Two diagnostic methods were used on each sample, a dipstick based method in which haemoglobin and myoglobin were separated by centrifugal filtration before the test and the modified Check4® Myoglobin protocol (see table 1). Samples were scored as either positive or negative for myoglobin by each method.

The results in all but one sample agreed for both methods. This sample was further diluted and it then gave a myoglobin positive result in agreement with the dipstick test.

More recently a false negative seen with an epileptic patient has resulted in a modification of the Check4® Myoglobin protocol to increase the secondary dilution in order to confirm true negatives.

Table 1 :Comparison of dipstick and immunochromatography assay protocols

| Test by haemoglobin dipstick | Modified Check4®-Urine Myoglobin 2-step assay |
|---|---|
| <p>1. The urine sample was first tested with a haemoglobin dip-strip. Negative samples were reported as <i>myoglobin not detected</i>.</p> <p>2. Positive samples were centrifuged to clarify if necessary, then 1-2ml were centrifuged at 5000rpm for 10 minutes through a 50kDa cut-off molecular filter. Myoglobin (MW 17kDA) passes through the filter whereas the larger haemoglobin molecule (MW 68kDa) is retained.</p> <p>3. The filtrate is tested for myoglobin with a haemoglobin dipstick.</p> <p>4. Positive results were reported as <i>myoglobin detected</i>. Negative results were reported as <i>myoglobin not detected;haemoglobin positive</i>.</p> | <p>Dilute the sample 1+5 with saline.</p> <p>Dispense 1 drop (20µl) of the diluted sample into well A.</p> <p>Add 5 drops (200µl) of diluent into well A</p> <p>Read the results on test cassettes after 5 and up to 10 minutes</p> |

IX- LIMITATIONS

- 1- As it is the case with any diagnostic procedure, the physician should confirm the data obtained using this test by other clinical methods.
- 2- Check4®-Myoglobin Test is designed to yield a positive result for myoglobin concentration at 100 ng/ml or higher. As no commercially available standard exists Check4® Myoglobin is quality controlled against bio-engineered protein obtained from human heart. The range of tested concentrations is 0-400ng/ml. No hook effect is seen at 400ng/ml, however urine concentrations can exhibit extremely high levels which may lead to a hook effect unless the dilution regime for negatives is adhered to.
- 3- Check4®-Myoglobin test only provides qualitative results.
- 4- Use only fresh urine specimens.
- 5-The diluent contains buffer however pH is important for sample stability and samples should be buffered as section V-sample collection.

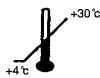
X- BIBLIOGRAPHY

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- (2)-Rosen CL et al. Early predictors of myoglobinuria and acute renal failure following electrical injury. J Emerg Med 1999; 17:783-789
- (3)-Dickson, J R. Crush injury. 2001.<http://www.mediccom.org/public/tadmat/training/NDMS/crush.pdf>
- (4)-Chen-Levy, Z, Wener, M H, Toivola, B, Daum, P, Reyes M & Fine J.S. Factors Affecting Urinary Myoglobin Stability In Vitro. Am J Clin Pathol 2005;123:432-438
- (5)-Hammond, J. Rapid confirmation of high levels of urine-myoglobin in crush injury victims. Path in Practice 2002;vol 3 issue 2:38-40

Explanation of symbols



Do not re-use



Store between



Consult Instruction for use



Expiry



For In-Vitro diagnostic use



GRJrev3.08