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CE

PATLab test

PATLab50 kit
PATLab100 kit
PATLab200 kit

COLORIMETRIC DETERMINATION OF BIOLOGICAL ANTIOXIDANT POTENTIAL

Principle

In blood, the defense against the noxious attack of unchecked reactive oxygen species (ROS) is performed by the plasma antioxidant barrier. This barrier includes exogenous (e.g. ascorbate, tocopherols, carotenoids, bioflavonoids, etc.) and endogenous (e.g. proteins, bilirubin, uric acid, cholesterol, GSH, etc.) compounds. Each compound is endowed with a specific antioxidant/reductive capability, creating together an antioxidant network that has the role to quench the oxidative action of the reactive species. The combined reductive capacity of the plasmatic compounds is associated with the ability of the plasma barrier to provide reducing equivalents (i.e. either electrons or hydrogen atoms) to the reactive species, avoiding the oxidation of organic substrates and the initiation of detrimental radical chain reactions, that lead to oxidative tissue damage.

PAT test is based on the ability of a colored solution, containing ferric (Fe^{3+}) ions, adequately bound to a special chromogenic substrate, to discolor when the ferric (Fe^{3+}) ions are reduced to ferrous (Fe^{2+}) ions. This principle can be observed by adding a reducing substrate, such as a sample of blood plasma.

Therefore, in the PAT test, the plasma sample to be tested is added to a colored solution that has been previously obtained by mixing a source of ferric (Fe^{3+}) ions (the R2 reagent) with a special chromogenic substrate (a thiocyanate-derived compound, the R1 reagent). After a short incubation (1 min), the solution will discolor, and the intensity of the color change will be directly proportional to the ability of plasma to reduce, during the incubation, ferric (Fe^{3+}) ions (initially responsible for the color of the solution).

By photometrically assessing the intensity of discoloration, the amount of reduced ferric ions can be adequately calculated, and the reducing ability or antioxidant capacity of blood plasma tested can be effectively measured. This antioxidant capacity is not absolute but relative to the tested substrate, i.e. ferric (Fe^{3+}) ions. Considering that ferric (Fe^{3+}) ions are naturally occurring components in biological systems, PAT test provides a reliable measure of the status of iron-reducing antioxidant capacity in a biological system.

Component list and storage instructions

R1 reagent	Chromogenic mixture containing thiocyanate		
R2 reagent	Ferric ion solution (Fe^{3+})		
Calibrator	Calibrator: Control Serum*		
Code	PATLab50 kit	PATLab100 kit	PATLab200 kit
R_1 reagent	1 x 50 ml	2 x 50 ml	4 x 50 ml
R_2 reagent	1 x 2 ml	2 x 2 ml	4 x 2 ml
Calibrator	1 x 1ml	1 x 1ml	1 x 1ml
* The concentration is specific for each lot; it is mentioned on the kit label and on the certificate of analysis			

Calibrator must be stored at 2-8 °C.
R1 and R2 reagents can be stored at room temperature. Store in a dark place.

Sample

PAT test can be carried out on fresh or heparinized plasma.
Do not use plasma treated with citrate or EDTA.

Control Serum: preparation, storage and use

The calibrator must be analyzed at room temperature; remove the vial from the refrigerator at least 30 minutes before executing the procedure. The bed absorbance value is applicable to all assays carried out with the same lot of reagents. The calibrator is stable by the expiration date when stored at 2-8 °C.

Working conditions and procedure

Wavelength	Optical path	Temperature
505 (500-510) nm	1 cm	37 °C

Follow the procedure steps outlined below:

- zero the instrument with distilled water
- prepare the working solution by mixing 40 µL of reagent R2 and 1 mL of reagent R1 - the solution will turn red
- read the absorbance of the working solution (Abs1*sample*)
- add 10 µL of plasma sample to the working solution and mix gently
- after one minute of incubation at 37°C, protected from light, make the second absorbance reading (Abs2*sample*)
- calculate ΔAbs*sample*: ΔAbs*sample* = Abs1*sample*- Abs2*sample*

Note: use the same procedure to calculate ΔAbs*blank reagent* by using distilled water as sample and ΔAbs*calibrator* by using calibrator as sample. These values are required in the calculation formula of the sample antioxidant capacity. They can be determined only once for each reagent lot.

Table 1. Components required for each procedure

	Sample procedure	Blank reagent procedure	Calibrator procedure
R1 reagent	1 mL	1 mL	1 mL
R2 reagent	40 µL	40 µL	40 µL
Sample	10 µL	-	-
Distilled water	-	10 µL	-
Control Serum	-	-	10 µL

Note: the quantities are indicative; they can vary and be adapted to the spectrophotometer specifications if needed, with the condition that the quantitative proportions between the reagents and between the reagents and sample stay the same as above.

The antioxidant capacity of the sample is expressed in U. Cor. (1 U. Cor = 1.4 µmol/l of Vit C) and calculated according to the following formula:

$$\frac{[\Delta Abs_{sample} - \Delta Abs_{blank\ reagent}]}{[\Delta Abs_{calibrator} - \Delta Abs_{blank\ reagent}]} \times [\text{calibrator}]$$

Where:
- ΔAbs = ΔAbs1-ΔAbs2 of the *sample/blank reagent/calibrator*
- [calibrator] is the calibrator's concentration, expressed as U Cor

Interpretation of results

Reference values (expressed as U. Cor.)	
>2800	High
2800-2200	Normal value
2200-2000	Borderline
2000-1800	Slight deficiency
<1800	Low

It is advisable that each laboratory determines its own reference values, particularly if samples are collected from species other than humans, such as birds or animals

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PAT test is useful in assessing the effectiveness of antioxidant therapies recommended in disease conditions associated with oxidative stress, and also as an effective screening tool for subjects in a state of apparent health.

References

Cornelli U et al. Pan Minerva Medica 2010; 52(2).
Cornelli U et al. Journal of cosmetics, dermatological Science and Applications; 2011 (1)
Benedetti S et al. Clin Lab Med (In press)