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d-ROMLab test

d-ROMLab50 kit
d-ROMLab100 kit
d-ROMLab200 kit

COLORIMETRIC DETERMINATION OF REACTIVE OXYGEN METABOLITES (ROMs)

Principle

Reactive Oxygen Species (ROS), also known as free radicals of oxygen are a class of compounds with an atom of oxygen that presents one or more unpaired electrons in its external orbitals. Due to their high reactivity, unchecked free radicals of oxygen have the potential to attack organic substrates, such as lipids, proteins, nucleic acids and carbohydrates, and generate hydroperoxides (R-OOH), a class of compounds also called Reactive Oxygen Metabolites (ROMs). Although ROMs are themselves oxidant molecules, they are relatively stable compared to ROS and, therefore, can be adequately detected and quantified.

d-ROMs test is based on Fenton's reaction, where in step 1 of reaction hydroperoxides (ROOH) from a biological sample react with iron (released from plasma proteins by an acid buffer - R2 reagent) and generate alkyl (R-O°) and peroxyl (R-OO°) radicals. In step 2, of reaction, the alkyl and peroxyl radicals oxidize an alkyl-substituted aromatic amine A-NH_2 (solubilized in a chromogenic mixture - the R1 reagent) and generate its oxidized form [A-NH_2°]$^+$, a pink colored compound, per following reactions:

1A) R-OOH + Fe^{2+} - *R-O° + Fe^{3+} + OH^- For alkyl radicals
1B) R-O° + A-NH_2 - *R-O^- + [A-NH_2*]$^+$

1C) R-OOH + Fe^{3+} - *R-OO° + Fe^{2+} + H^+ For peroxyl radicals
1D) R-OO° + A-NH_2 - R-OO^- + [A-NH_2*]$^+$

The color change that occurs as a result of oxidation of the aromatic amine A-NH_2 is photometrically quantifiable by means of a spectrophotometer. The intensity of color change in the sample solution is directly proportional with the concentration of ROMs in that sample, according to Lambert-Beer's law.

Component list and storage instructions

R1 reagent	Chromogen mixture (alkyl-substituted aromatic amine)		
R2 reagent	Acetate Buffer (pH 4,8) preservatives and stabilizers		
Calibrator	Calibrator: Control Serum*		
Code	d-ROMLab50 kit	d-ROMLab100 kit	d-ROMLab200 kit
R_1 reagent	1 x 0.5 ml	2 x 0.5 ml	4 x 0.5 ml
R_2 reagent	1 x 50 ml	2 x 50 ml	4 x 50 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			

R1 and R2 reagents and calibrator must be stored in a dark place, at 2-8 °C.

Sample

d-ROMs 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

KINETIC MODE

Follow the procedure steps outlined below:

- zero the instrument with distilled water
- prepare the working solution by mixing 10 µL of reagent R1 and 1 mL of reagent R2
- add 10 µL of plasma sample to the working solution and mix gently
- incubate for 3 minutes at 37°C, then proceed with the first absorbance reading (Abs1*sample*)
- after 2 more minutes of incubation time at 37°C, proceed with second absorbance reading (Abs2*sample*)
- calculate ΔAbs*sample*: ΔAbs*sample* = Abs2*sample*- Abs1*sample*

Note: use the same procedure to calculate ΔAbs*calibrator* by using calibrator as sample. This value is required in the calculation formula of the d-ROMs testf the sample. It can be determined only once for each reagent lot.

Table 1. Components required for each procedure

	Sample procedure	Calibrator procedure
R1 reagent	10 µL	10 µL
R2 reagent	1 mL	1 mL
Sample	10 µL	-
Control Serum	-	20 µL*
*The value reported in the certificate of analysis correlates with the use of this quantity		

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 result of d-ROMs test is expressed in U. Carr. (1 U. Carr. = 0.08 mg/dl of H_2O_2) and calculated according to the following formula:

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

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

Interpretation of results

Reference values (expressed as U. Carr.)	
250-300	Normal value
301-320	Borderline to oxidative stress
321-340	Slight oxidative stress
341-400	Oxidative stress
<400	High oxidative stress

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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d-ROMs test is useful in assessing the pro-oxidant status in biological systems, as an early marker of inflammation and as a reliable indicator of systems' response to environmental changes. It is also an effective screening tool for subjects in a state of apparent health.

References

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