Allergy, 1991, 46,

A sensitive spectrophotometric diamine oxidase activity assay in platelet rich plasma

Dear Sir,

Koller et al. describe in their letter to Allergy an *"Inaccurate assessment of diamine oxidase activity in platelet-rich plasma"* and relate their measurements to our original report *"Monoamine and diamine oxidase activities in atopic eczema"* in Allergy 43, 318-319, 1988.

1. In previous direct conversations we explained to Dr. Koller that we are not measuring activities in plasma - for which our assay is not sensitive enough - but in *platelet-rich plasma*.

   To obtain this platelet-rich plasma it is very important to centrifuge blood for 10 min at the correct speed (53 *g* or 600 rpm); at a higher speed no activity will be seen because of platelet sedimentation.

   Fresh *EDTA-blood* (EDTA is necessary to prevent degradation of the enzymes by proteases) must be used for isolation of platelet-rich plasma and the suspension must be used immediately because DAO activity decreases within hours *in vitro*. A reduction in DAO activity of a few percent by the EDTA in our samples is without significance, since we relate patient measurements to control samples.
2. The 10 min Triton X-100 incubation time has to be completed to release DAO from the platelets. Absorbance at 502 nm by 15 to 25 min (linear ascent) was recorded. Absorbance at the beginning of Triton X-100 incubation decreases because of the disappearing turbidity, the resultant *ascending linear curve* has been used for activity measurements. The measurements can be repeated shortly after without considerable changes in extinction. The DAO activities are also reproducibly stimulated by their cofactor vitamin B_6 (not published).
3. The peroxidase buffer (peroxidase: Boehringer 413470) and the 2', 7'-dichlorofluorescein diacetate (Serva 19353) solution must be protected from light and renewed every 8 to 10 weeks.

In the meantime we have measured DAO-activities in more than 500 patients and over 100 control samples (in the same range as originally reported). We strongly support the view that the method, originally described by Köchli & Wartburg (Anal Biocham *84*, 127-135, 1978) and modified by us for determination of DAO activity in platelet-rich plasma, is a radioactive-free, sensitive spectrophotometric assay for this purpose.

If the above details and arguments are not enough, than we gladly invite Dr. Koller and colleagues to check the DAO measurements and calculations in our laboratory in Germany.

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