

## Letter to the Editors

### Degradation of serum neopterin during daylight exposure

*Keywords:* Neopterin; Stability; Light exposure; Storage

2-Amino-4-oxo-6-(D-erythro-1, 2, 3, trihydroxypropyl)-pteridine (Neopterin) and its reduced form, 7,8-dihydroneopterin, are produced in large amounts by interferon- $\gamma$ -activated monocytes/macrophages. Therefore, the monitoring of neopterin concentrations in body fluids is a sensitive way to detect Th-1 type immune response [1,2]. Consequently, measurement of neopterin concentrations in urine, serum or cerebrospinal fluid are employed as a laboratory diagnostic tool, e.g. to earlier detect immunologic complications in transplant recipients [3] or to predict prognosis in HIV infection and malignancy [4–6]. Neopterin concentrations are also increased during acute virus infections, and increased neopterin concentrations are observed in the majority of patients before antibody seroconversion becomes detectable. Therefore, in Austria, blood donations are screened for elevated neopterin concentrations since 1994. Donations with neopterin  $>11$  nmol/l are excluded from transfusion. Neopterin concentrations in serum, plasma and cerebrospinal fluid specimens are usually determined by immunoassays [7–11]. Frequent thawing and freezing cycles are known to influence neopterin concentrations in serum [7]. Furthermore, it is well known that neopterin in aqueous solutions and in urines is degraded by direct sunlight exposure [12], but this effect has never been studied in detail in serum specimens. We examined the stability of neopterin in sera kept under various laboratory conditions, with and without daylight or direct sunlight exposure.

For the first out of three series of experiments, random serum samples stored at  $-20$  °C were pooled and aliquoted (0.5 ml fractions) into transparent, capped LP4 plastic tubes (Eppendorf, Hamburg, Ger-

many). Duplicates of aliquots were immediately frozen and kept at  $-20$  °C as controls. To study effects of light exposure, samples were (i) stored at  $+4$  °C in the dark, (ii) exposed at room temperature on the lab-bench but shielded from direct daylight, or (iii) placed next to a window and (iv) exposed to direct sunlight. The exposure time of the aliquots was 0, 4 and 6 days, respectively. Upon the end of the exposure period, all aliquots were frozen again and stored at  $-20$  °C in the dark until, on day 7, the ELISA (Brahms Diagnostics, Berlin, Germany) was performed.

In a second series of experiments, a fresh serum preparation and two lots of frozen serum were used. Fresh serum was prepared by allowing 50 ml of fresh blood to clot for 3 h at room temperature in the dark. After centrifugation for  $2 \times 15$  min at 3000 rpm and  $20$  °C, the supernatant was removed, aliquoted (200  $\mu$ l) into capped, transparent LP4 plastic vials kept at  $4$  °C in the dark overnight until the experiment was started. Frozen serum aliquots of known neopterin concentrations were mixed to give a serum pool of high-normal neopterin concentration (8.8 nmol/l) and a serum pool of average-normal neopterin concentration (5.44 nmol/l). Upon aliquotation into capped LP4 transparent plastic vials, fractions were stored in the dark at  $-20$  °C until the experiment was started. The next day, samples from  $+4$  °C were removed and exposed for 0–6 h at  $+7$  °C to neon-light (cold-room with lights switched on) or at room temperature in indirect daylight next to a window. Zero-controls were left at  $+4$  °C in the dark. Similarly frozen high- and low-neopterin samples were exposed for 0–6 h at  $+4$  °C in darkness or at room temperature next to a window in direct daylight. Controls were kept at  $-20$  °C throughout. The time course was performed

in the way that samples exposed for 6 h were removed first from storage and samples exposed for 1 h last. As the neopterin ELISA was started right after exposure times were completed, no additional freezing and thawing of the samples was required.

In the last series of experiments, neopterin degradation in fresh and frozen serum was compared. Frozen serum was taken from  $-20^{\circ}\text{C}$  and pooled. All sera were aliquoted ( $200\ \mu\text{l}$ ) into LP4 plastic vials frozen and exposed at  $+7^{\circ}\text{C}$  in the dark or in neon-light, and at room temperature in the dark or next to a window in direct daylight. After exposure times of 0–12 h, all samples were stored at  $-20^{\circ}\text{C}$  in the dark until the ELISA was performed the following day.

All experiments were run in triplicates. To determine serum neopterin, an ELitest<sup>®</sup> Neopterin Screening ELISA from BRAHMS Diagnostics was used. The kit was applied according to the manufacturer's protocol. The minimal neopterin concentration detectable was 2 nmol/l.

In all experiments performed, exposure of serum to direct daylight resulted in rapid degradation of neopterin. The average half life of serum neopterin in direct daylight was 3.5–4 h. No difference in neopterin degradation was detected between fresh and aged serum or between serum containing higher or lower initial neopterin levels (second series, data not shown). Fresh serum was prepared shortly before the experiments were started. Aged serum had been stored

for 3–6 months at  $-20^{\circ}\text{C}$  in the dark and was frozen and thawed twice.

When aged, serum (neopterin concentration = 12.4 nmol/l) was kept at room temperature but not directly exposed to sunlight (i.e., shielded behind a cupboard), neopterin was more stable, with 89% and 78% of the initial neopterin concentration still being detectable after 4 and 6 days, respectively. When the same serum was stored at  $+4^{\circ}\text{C}$  in the dark for 4 or 6 days, neopterin was still 94% or 92% of the starting concentration. Storage for up to 6 days at  $-20^{\circ}\text{C}$  in the dark did not alter the neopterin concentration (Fig. 1).

Both aged and fresh serum stored at  $+7^{\circ}\text{C}$  in the cold-room, either in the dark or at neon-light, or kept in the dark at room temperature ( $25^{\circ}\text{C}$ ) showed no significant decrease of neopterin during a 12-h testing period. Fresh neopterin serum (4.5 nmol/l at time point 0) reached the lower limit of detection of the ELISA used (2 nmol/l) within 4 h of exposure (Fig. 2a). In addition, the aged serum, neopterin concentration = 8.5 nmol/l at time point 0, dropped to less than 30% within 4 h of exposure to direct daylight (Fig. 2b).

As known from previous investigations [9], neopterin is susceptible to degradation when exposed to direct sunlight, as was demonstrated in urine and in aqueous standard preparations. In the present study, similar results were found for serum neopterin regardless whether fresh or aged serum was tested. The

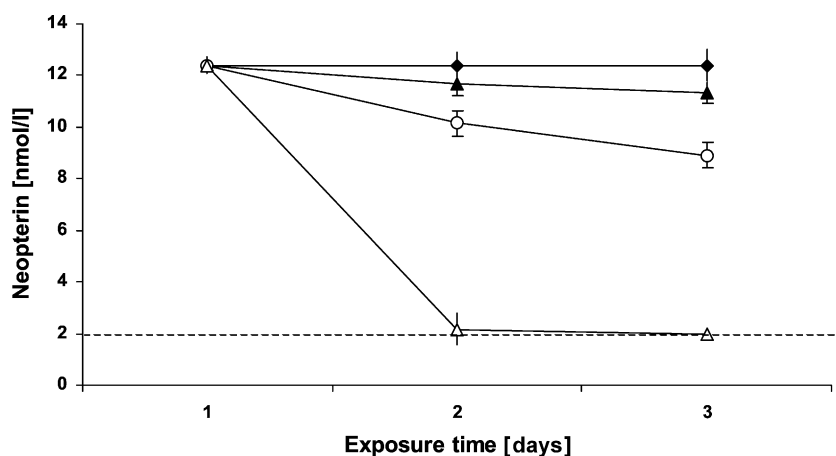


Fig. 1. Pooled serum samples were exposed for 0, 4 and 6 days at room temperature (RT) in vicinity to a window to direct sunlight ( $\Delta$ ) or protected from direct sunlight ( $\circ$ ). Storage at  $+4^{\circ}\text{C}$  ( $\blacktriangle$ ) or  $-20^{\circ}\text{C}$  ( $\bullet$ ) both in the dark was performed in addition. The detection limit of the neopterin ELISA applied was 2 nmol/l (all measurements were run in triplicates, data points showing mean values  $\pm$  S.E.M.).

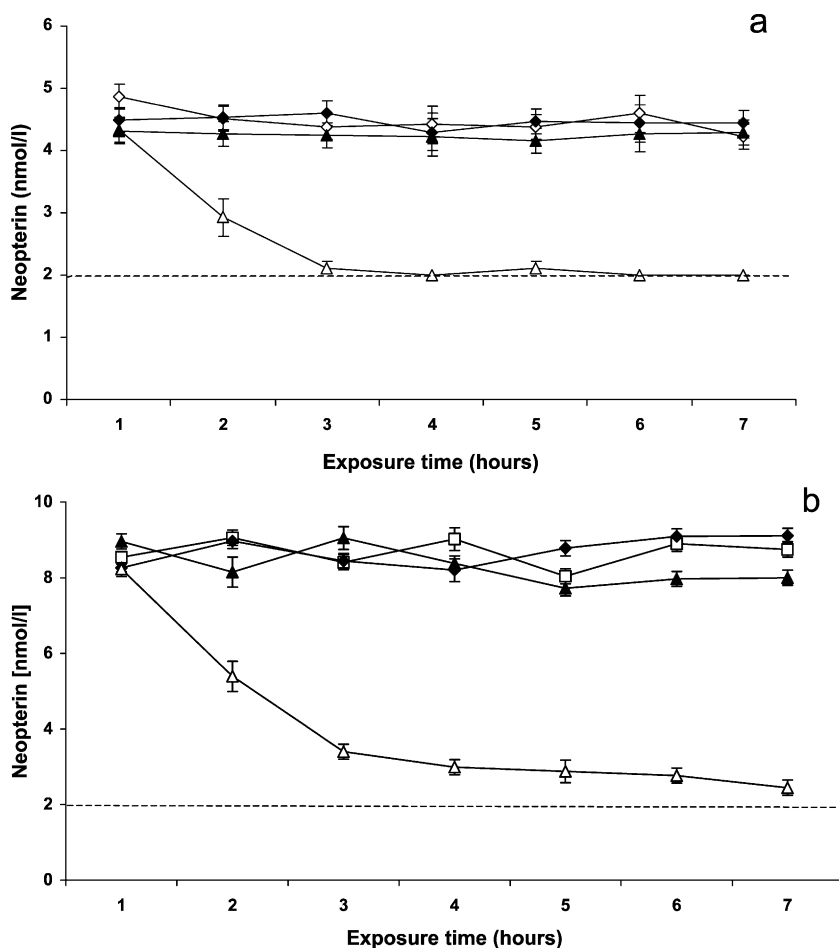


Fig. 2. Fresh (a) and aged (b) serum samples were exposed for 0–12 h in vicinity to a window to direct sunlight at room temperature ( $\Delta$ ) or stored in the dark ( $\blacktriangle$ ). In addition, exposure at  $+7^\circ\text{C}$  to artificial (neon) light ( $\square$ ) or in darkness ( $\blacklozenge$ ) was performed. The detection limit of the neopterin ELISA applied was 2 nmol/l (---).

implications for daily routine diagnostics are strict handling of serum samples to be tested for neopterin: it is absolutely important to avoid sample exposure to daylight during all determination procedures. As it was found that short-time exposure to ambient temperature does not cause significant decrease of serum neopterin concentrations, sample handling according to common GLP or GMP rules will not cause any problems. Routine laboratory procedures can therefore be performed without any measurable influence on neopterin concentrations at room temperature, at indirect daylight or under artificial (neon) light. Working with, or even short-time storage of samples in direct vicinity of a window must be avoided. Thus,

also pipetting of specimens or aliquotting in vicinity to an unprotected window has to be avoided. In blood donation settings, collecting samples and measuring neopterin concentrations are often performed at different sites and samples often have to be transferred from one location to another. During transport, protection of these samples from direct daylight has to be guaranteed. If these recommendations are not kept, significant decline of neopterin concentrations will take place, which, especially in the blood transfusion settings, reduces the sensitivity of neopterin monitoring to detect acute virus infections.

Regarding the storage of serum samples: as demonstrated in the present study, short-time storage at

+7 to +4 °C in the dark or under artificial light (cold-room) has no effect on serum neopterin. For protection from sunlight, dark containers or tubes are recommended. For long-term storage,  $\leq -20$  °C in the dark is strictly necessary.

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