Neopterin – Analysis in fecal and colon samples

Neopterin is a valuable early biochemical marker of cellular immunity. It is synthesized by macrophages upon stimulation with the cytokine interferon-γ. Increased production of neopterin in humans is indicative for an activated cellular immune response and neopterin levels are of importance in the pathogenesis and progression of various human diseases.

The GenWay Biotech Inc. Neopterin-ELISA is an enzyme immunoassay for the quantitative determination of neopterin in human plasma, serum and urine. Preparation of other specimen like fecal (stool) or colon dialysate is not based on validated data so far. Such new applications need to be adapted and validated for the specific use.

This application note summarizes existing studies in the field of intestinal-neopterin research. Several studies demonstrate the possibility to measure neopterin in fecal or colon samples. The intestinal system encounters many infectious agents or injurious substances evoking a cellular immune response. Thus, neopterin concentrations provide prognostic information in patients with inflammation of the colon.

Campbell et al studied the influence of the intestinal parasite *Giardia lamblia* in Gambian children. They found out that fecal neopterin concentrations were associated with *Giardia* and inversely associated with growth. This was consistent with the hypothesis that intestinal inflammation in tropical infants may impair growth. They conclude that also factors other than *Giardia* are causing enteropathy and growth failure in Gambian infants, making fecal neopterin a useful marker for clinical disease states.

Nisreen et al found out that fecal neopterin was significantly higher in patients with ulcerative colitis, especially among those with active disease. Patients with ulcerative colitis, either active or in remission, could not be distinguished from controls when looking at urine neopterin. Different serum neopterin pattern did not show correlation suggesting that several mechanisms are involved in colitis ulcerosa.

Ledjeff et al studied neopterin levels in colon dialysates of healthy individuals and in comparison, with individuals under a starvation diet and with patients. In parallel, urinary and salivary neopterin levels were determined. The mean neopterin concentration in colon dialysate was about 10 times higher than in serum and saliva; urinary neopterin was 200 times higher. These high neopterin values agree with a great impact of the cellular immune system within the human colon. They conclude that common diseases like acute gastritis had a strong and prompt effect on the neopterin levels in colon dialysate. The lower neopterin levels in people with a starvation diet may argue for a regeneration of the cellular immune system during fasting.

Because this procedure for colon dialysates could not be used as a screening test, the same group from Fuchs et al developed and evaluated a stool collecting device for the measurement of stool water. Highly elevated fecal water neopterin concentrations were found in chronic inflammatory bowel diseases.
Pre-analytical informations: fecal sample handling

Generally, fecal samples from patients are extracted and diluted before performing the immunoassay. In the literature the following procedures are recommended for handling with fecal samples:

**Sample collection**
The usual methods for collection and treatment of fecal samples are acceptable. Specialized sample devices are named to ease the collection, stabilization, homogenization and shipment of fecal samples (a sample of the size of a pea is sufficient) (ScheBo ®• E1 Quick-Prep, Cat.-No. 07-Quick, Roche Diagnostics Cat.-No. 745804). As an alternative, fecal samples directly can be collected and weight with polypropylene devices.

**Sample pretreatment**

1. Weigh ca. 0.1 – 0.2 g fecal sample (the exact amount of sample has to be documented) dilute in 1 mL of sample diluent (mix carefully).
2. Shake suspension resp. sample device for 30 min to get an optimal mixture with the extraction diluent.
3. Centrifugate suspension for 20 min at 3500 Upm (fecal sample extract).
4. The proposed shelf life for fecal samples is 3 days for storage at 4 – 8 °C and 1 year for -20 °C. Fecal sample extracts can be stored 1 day at 4 - 8 °C (1 month at -20 °C).
5. Dilutions of extracts should be done directly before assaying. No additional stabilizers should be added!

**Assaying:**
- Supernatant (sample extract) has to be diluted by dilution buffer
- **10 µl** diluted supernatant (sample) will be used in the test

**Calculation:**
- Results are expressed in nmol/L
- For conversion to ng/mL use multiplication factor 0.253
- Multiply with dilution factor (factor 10 x Einwaage) (= nmol/g)

**Notes for handling and storage:**
- Sampling should be done according to standardized procedures
- Use sampling devices with polypropylene (PP)
- Keep samples away from heat or direct sun light
- Avoid repeated freeze-thaw cycles
- Dilutions should be done directly before assaying