Quantitative determination of hemoglobin in stool

Determination of hemoglobin in stool

Quantitative determination of hemoglobin in stool is the most accurate method for determining occult bleeding, a method suitable for screening colorectal tumors. The hitherto used guaiac Haemoccult test - gFOBT is less sensitive, influenced by diet and in many countries it is replaced by the immunochemical test - iFOBT, which demonstrates hemoglobin by means of rapid monoclonal antibody tests. Quantitative determination of hemoglobin correlates with the bleeding rate of precancerous lesions (adenomas) and colon tumors. Studies in recent years have tested several immunochemical analyzers for the quantitative determination of blood in the stool, most of which are Japanese-made. ROC (Receiver Operating Characteristic) curves show 95.3% specificity for advanced adenomas at a sensitivity of 100 ng Hb/mL. Comparison of gFOBT and iFOBT by the OC Sensor method shows 3 times greater detection of advanced adenomas and carcinomas by the iFOBT method.

Quantitative determination

Quantitative determination of human hemoglobin in faeces is based on agglutination, latex reaction with antibody to A0 human hemoglobin. The method determines hemoglobin using a polyclonal antibody to Hb A0 IgG - i.e. with a multiple monoclonal antibody. (The monoclonal antibody reacts with only one epitope, the polyclonal antibody normally reacts with different epitopes, the antibody for this analysis requires a multiple reaction for agglutanation activity, i.e. a multiple monoclonal reaction). The analysis itself is performed by turbidimetric measurement at 600 nm, the calibration is usually several points in the range of 20–2000 ng Hb/ml. Quantitative analysis allows, in contrast to qualitative - rapid tests - to define the optimal cut-off value.

Collection system

The stool collection system provides a quantitative aspect of stool collection per 10 mg sample, stool extraction in 2 ml of stabilizing buffer, analyte filtration, and direct collection of the extract into the analyzer reaction cuvette. An example is the sampling system for the OC-Sensor analyzer (Eiken, Japan). The collected stool (20–200 mg) on the collection brush is pushed through a precisely defined septum after insertion into the cassette, which ensures the extraction of 10 mg of stool (with approx. 10% error) in 2 ml of stabilizing damper. In the analyzer, the cartridge is pressed by mechanical pressure and the stool extract is forced through the filter into the injection well under the aluminum foil. This foil is perforated and a spray of 25 µl of the extract is applied to the measuring cuvette with a needle.

Sources

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