Expert Working Group – 'FIT for Screening'

Short Discussion Document No. 3 – Stability of haemoglobin

Prepared by: Prof Stephen Halloran, Dr Helen Seaman and Dr Michelle Young,
Bowel Cancer Screening Programme Southern Hub, Guildford, UK (e-mail: s.halloran@nhs.net)

1. Case for change

The application of faecal samples to absorbent paper and drying before analysis confers relative stability upon haemoglobin (Hb) in faeces (Young, 1996) and therefore upon guaiac occult blood tests (gFOBT).

gFOBT detect the presence of the chemically robust iron-containing pyrrole ring, the haem component of Hb. Faecal immunochemical tests for Hb (FIT), clinically superior tests, detect the protein globin in Hb and, in quantitative FIT, the amount of Hb is also measured. FIT confer the opportunity for analytical specificity for *human* Hb but carry vulnerability due to globin's susceptibility to degradation, both in the colon and in the test device. Hb in native faeces, once passed, is very unstable (Brown and Fraser, 2008). Vulnerability to degradation may be further confounded by transporting the faecal sample wet in aqueous solution.

The screening community, both FIT device manufacturers and clinical users, is faced with two challenges: (1) how to minimise the rate of Hb degradation between collection and analysis and (2) how to express FIT system stability characteristics in a way that has practical value to the user and enables systematic and accurate comparison between products.

Without the adoption of guidelines that facilitate a standardised approach to assessing the stability of faecal Hb in FIT devices, screening programmes risk criticism for clinical negligence and unnecessarily missed cancers. Moreover, manufacturers risk being accused of misleading their customers.

We need an open and systematic approach to assessing and reporting stability that includes detailed and explicit information about the method used. The heterogeneous nature of faeces presents a challenge to manufacturers and users alike and we are likely to have to settle upon an imperfect but standard approach that enables comparison between products.

As population-based screening is being adopted across the globe, we need the commercial sector and clinical professionals to work together to develop agreed guidelines on FIT stability - improving it, assessing it and reporting it.

2. Proposed solution

a) No manufacturer should make the statement that 'the sample [or the Hb] is stable for …. days' - this is both inaccurate and misleading since Hb will continue to degrade from the time of bleeding into the gastrointestinal tract.

b) Develop two distinct and complementary assessment methods that can be used by manufacturers to determine Hb stability in their products:

- Stability in an artificial but standardised matrix that mimics faeces.
- Stability in faecal samples that meet specified properties and are treated in a prescribed manner.

c) A standardised method for assessing the bactericidal effectiveness of a preservative buffer.

d) Identify QA stability monitoring methods that manufacturers should adopt as part of GMP (Good Manufacturing Practice).

e) Identify QA methods for screening programmes to adopt to monitor FIT stability.

f) The guidelines should describe each method in sufficient detail so as to enable it to be reproduced in different laboratory settings. They should describe materials and reagents, the number of samples and measurement required and the method of statistical analysis.

g) The outcome of the stability assessments should be described in product literature in a form that enables simple but reliable performance comparisons to be made.

h) The limitations of methods advocated by the EWG should be clearly described in the guidelines.

i) The EWG should seek endorsement of the guidelines by the WEO and other relevant national and international professional and regulatory organisations.

j) The EWG should encourage research that explores the process of faecal Hb degradation, its effect on FIT measurement and methods to improve stability.

3. Issues for consideration

Haemoglobin begins to degrade from the time of bleeding into the gastrointestinal tract. The rate of degradation in faeces, once passed, will be influenced by:

a) the period between collection and analysis (elapse time),

b) the temperature at which the sample is transported/stored,

c) the properties of individual faecal samples including bacterial flora and chemical composition (pH, ionic strength, specific molecules),

d) the adsorptive properties of faecal particulate matter,

e) the preservative properties of the device/buffer, and

f) other, as yet unknown, factors that might be significant.

Hb and some of its breakdown products might remain in the faeces but be inaccessible to some analytical methods due to irreversible adsorption to the faecal matrix.

Antibodies used in the FIT assay might cross-react with some of the larger peptides produced by Hb degradation.

Antibodies used in the FIT assay might cross-react with proteins/peptides that are not derived from Hb and they might degrade more slowly or more quickly than Hb.

The use of monoclonal versus polyclonal antibodies is likely to change the performance of the test and, depending on the antigenic epitope(s), might change the FIT stability.

Identification of a standardised artificial faecal matrix will provide the opportunity to produce comparative descriptive product and performance information. Although an artificial matrix will have implicit limitations and will need to complement other means of assessment, it will provide the opportunity for standardised assessment across manufacturers, products and laboratories.

Critical temperatures and time periods for stability assessment need to be identified.

The effect of freezing needs to be evaluated since one product has already shown that faecal Hb degrades at temperatures less than $0^{\circ}C$.

4. Relevant publications

Allison JE. The role of faecal occult blood testing in screening for colorectal cancer. Pract Gastroenterol 2007;31:20-32.

Bron LF, Fraser CG. Effect of delay in sample on haemoglobin determined by faecal immunochemical tests. Ann Clin Biochem 2008;45:604-5.

Grazzini G, Ventura L et al. Influence of seasonal variations in ambient temperatures on performance of immunochemical faecal occult blood test for colorectal cancer screening: observational study from the Florence district. Gut 2010;59:1511-15.

Guittet L, Guillaume E et al. Analytical comparison of three quantitative immunochemical faecal occult blood tests for colorectal cancer screening. Cancer Epidemiol Biomarkers Prev 2011;20:1492-1501.

Rabeneck L, Rumble RB et al. Fecal immunochemical tests compared with guaiac fecal occult blood tests for population-based colorectal cancer screening. Can J Gastroenterol 2012;26:131-47.

Van Roon AHC, Hol L et al. Are fecal immunochemical test characteristics influenced by sample return time? A population-based colorectal cancer screening trial. Am J Gastroenterol 2012;107:99-107.

Young GP, Sinatra MA, St John DJ. Influence of delay in stool sampling on fecal occult blood test sensitivity. Clin Chem 1996;42:1107-8.