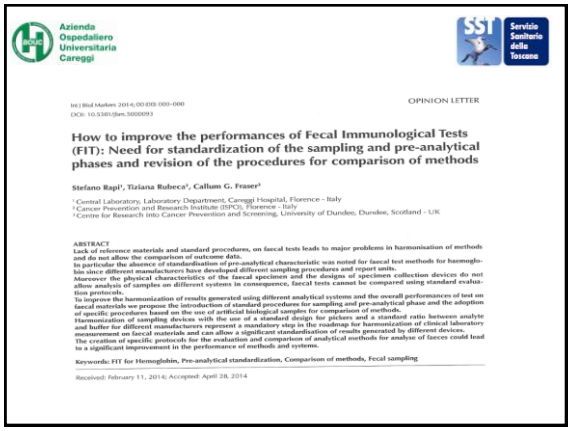

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OPINION LETTER

How to improve the performances of Fecal Immunological Tests (FIT): Need for standardization of the sampling and pre-analytical phases and revision of the procedures for comparison of methods

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ABSTRACT
Lack of reference materials and standard procedures, on faecal tests leads to major problems in harmonisation of methods and do not allow the comparison of outcome data.
In particular the absence of standardisation of pre-analytical characteristic was noted for faecal test methods for haemoglobin since different manufacturers have developed different sampling procedures and report units.
Moreover the physical characteristics of the faecal specimen and the designs of specimen collection devices do not allow analysis of samples on different systems in consequence, faecal tests cannot be compared using standard evaluation protocols.
To improve the harmonization of results generated using different analytical systems and the overall performances of test on faecal materials we propose the introduction of standard procedures for sampling and pre-analytical phase and the adoption of specific procedures based on the use of artificial biological samples for comparison of methods.
Harmonization of sampling devices with the use of a standard design for pickers and a standard ratio between analyte and buffer for different manufacturers represent a mandatory step in the roadmap for harmonization of clinical laboratory measurement on faecal materials and can allow a significant standardisation of results generated by different devices.
The creation of specific protocols for the evaluation and comparison of analytical methods for analyse of faeces could lead to a significant improvement in the performance of methods and systems.

Keywords: FIT for Hemoglobin, Pre-analytical standardization, Comparison of methods, Fecal sampling

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Standardisation of collection devices for faecal material for the detection of haemoglobin with immunochemical tests.

- **The aim of the project is to further facilitate the harmonization of analytical results from faecal immunochemical tests for haemoglobin (FIT) in faecal materials irrespective of the sampling strategies.**
- **The project involves documentation of the 'state of the art' and implementation of the development of a detailed protocol for the standardization of the pre-analytical phase that will be adopted at a European level in order to produce a regulatory action relating commercially available sampling collection devices in the European Community.**

Objectives to be achieved, results expected:

Production of guidelines for the standardisation of methods produced by manufacturers, particularly the development of devices for sampling of faecal material to be used within the European Community.

- **In detail:**
  Determination of the most appropriate design and characteristics of the sample collection device (design, dimensions and choice of the most suitable material for the sampling probe) to reduce the differences in the amount of material collected.
- **Determination of the relationship between faecal material and buffer in the sample collection device. Production of a prototype of the sample collection device and its experimental verification with different methods for the quantitative determination of faecal haemoglobin.**

Materials and methods:

- Reconnaissance phase:overview of current sampling systems.
  Literature search. Acquisition of products from the companies, contact with product manufacturers.
  Experimental phase: Evaluation of the analyte/volume of buffer relationships for the constancy of the analyte.
- Development of novel techniques for the assessment of analyte/volume.
  A series of samples obtained by adding Hb to faecal material will be analysed in a serial manner with at least 2 commercially available FIT.
  Step Reprocessing: Choice of optimal faecal material / volume of buffer ratio and design of a standard sample collection device.
- Creation of a prototype of the faecal material sample collection device to introduce into the European Community. Verification of the device with different FIT

Definition of the final model in collaboration with the Joint Research Centre and creation of mandatory standards to be used within the European Community.

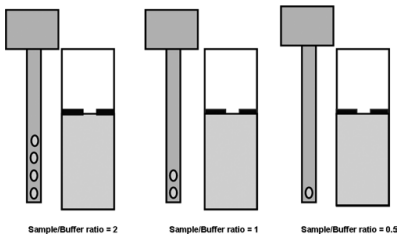

Fig. 2 - *Example of a standardized design for probe/pickers for fecal specimen collection and a suggested relationship between sample size and buffer volume to reduce the overall specimen collection variability.*

Collaborations planned:

Creation of a European inter-corporate study group including societies of Laboratory Medicine and Scientific Societies involved in Screening Programmes. The study group should ensure the maximum adoption of the new device among the European Community and come to the creation of approved Guidelines for both the pre-analytical phase and the analytical testing of faecal material.

Revision of the procedures for comparison of methods

All analytical systems for fecal tests are designed to work with the related sampling devices and, inter alia, ionic strength and pH of the material sampled for analysis, as well as analytical working ranges, are closely related to the specific system.

The introduction of specific protocols for comparison of methods of fecal analysis and the separate investigation of the pre-analytical and analytical phases could be useful to address these issues.

We suggest the introduction of 2 procedures that somewhat vary from the standard protocols normally used for comparison of methods.

Both procedures adopt the use of artificial biological samples (ABS) obtained by adding different amounts of analyte to native fecal specimens with no detectable analyte.

1st procedure

A standard set of ABS need to be generated using the specimen collection devices of both candidate and reference systems.

The sets are analyzed with both systems using the appropriate specimen collection device and the solutions are then withdrawn from the devices, pipetted into sample cups and then analyzed with the other system.

The complete sets of samples should be analyzed in duplicate on both systems and statistical analysis of the data should be performed as described in the standard NCCLS procedure for methods' comparison.

The aim of this procedure is the generation of data on the concordance between 2 methods, the evaluation of the comparative bias in the analyte measurements from different systems, and the investigation of the effect of specimen collection devices on the measurements.

2nd procedure

A large series of ABS that would result as positive or negative should be analyzed on both systems.

Only the appropriate specimen collection device should be analyzed on the correspondent system, in order to evaluate the effects of the sampling strategy on analytical results according to the characteristics of the specimen collection devices. The data obtained should be investigated by linear regression, Pearson's correlation coefficients, and Bland Altman analysis.

A 2 x 2 contingency table can be built using the same data to simulate the diagnostic assessments on the material analyzed. The presence or absence of analyte in the ABS is used as reference, and the cutoff suggested by the manufacturers are used in the assessment of diagnostic accuracy.

Preliminary information on sensitivity and specificity of methods can be obtained from these data.

"SIBioC Tumor Markers WG" meeting agenda october 14 Rome

- Aggiornamento stato di avanzamento del progetto 'Guida' (M. Gion)
- Aggiornamento sui lavori del 'Tavolo Interregionale Biomarcatori' (M. Gion)
- Presentazione del progetto sulla 'Standardizzazione dei dispositivi di prelievo del materiale fecale (S. Rapi)
- Proposta per la creazione di un gruppo di lavoro per l'Elaborazione di protocolli di indagine per i test su materiale fecale (S. Rapi)
- Varie ed eventuali

Proposal for a WG for the Revision of the procedures for comparison of methods on faecal materials