Editorial



# Is it possible to correctly assess the pre-analytical characteristics of faecal tests?

**Stefano Rapi[1], Filippo Cellai[2], Tiziana Rubeca[2]**

[1]Central Laboratory, Service Department, Careggi Hospital, Florence, Italy; [2]Cancer Prevention and Research Institute (ISPRO), Oncological Network, Prevention and Research Institute, Florence, Italy

*Correspondence to:* Dr. Stefano Rapi. Central Laboratory, Service Department, AOU Careggi, Largo Brambilla 3, 50139 Florence, Italy, Email: rapis@aou-careggi.toscana.it.

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## Introduction

ISO 15189 (Medical laboratories—Requirements for quality and competence) requires the sources of variation to be assessed in all phases of the examination process. "Section 5.5.1.4. Measurement uncertainty of measured quantity values" requires the laboratory to "determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples" (1). The laboratory shall define the performance requirements for the measurement uncertainty of each measurement procedure and regularly review estimates of uncertainty measurement.

Moreover, the inclusion of pre-analytical imprecision in the measurement uncertainty estimate may be a direct consequence of the note 1 in the same section, that "relevant uncertainty components are those associated with the actual measurement process, commencing with the presentation of the sample to the measurement procedure and ending with the output of the measured value".

Therefore, the evaluation of the imprecision arising from sample collection and handling falls directly within the responsibilities of laboratories. However, up until the present, this has not been well studied or documented for all, or at least the major components, of examinations on faecal material.

In the document "Guidance for faecal occult blood testing" (2) the First Level Working Group of GISCoR (Gruppo Italiano Screening Colorettale) attempted to delineate practical solutions to improve the quality and governance on the faecal haemoglobin concentrations (f-Hb) generated using faecal immunochemical test (FIT) for haemoglobin used in colorectal cancer (CRC) screening programmes. However, considering the state of the art of quantitative methods currently available on the market, many unsolved questions require to be addressed and a comprehensive revision of sampling strategies needs to be performed to improve the quality of the pre-analytical phase of faecal material examinations so as to conform to the ISO 15189 requirements.

## Characteristics of collected faecal material

A great difference can be observed in both texture and relative density of samples of faecal materials. Texture differences were addressed by use of the Bristol "stool hardness" scale (3) but this information probably only has clinical interest and is rather irrelevant to the laboratory setting. In contrast, information arising from the weighing of the faecal material is more significant from the aspects of examination point. Significant differences in the relative density can be observed in samples of the same Bristol scale number, and also in samples from a single bowel motion of the individual. However, this evidence, easily observed, must be relevant in the clinical setting, in evaluation of the results of examination of faecal material.

Although the weighing of samples is difficult and time-consuming, some laboratories, in particular those involved in research and microbiome analysis, use this strategy to tie together objective estimates of the sampling with the data reporting in the post-analytical phase. Pre-analytical systems which are able to prepare a weighted sample, although not widely used, are available on the market (4). It

should be remembered that, in this approach, only the use of weight of measurand per weight of faeces is appropriate for the data reports.

Following the paper of 2012 by Expert Working Group on FIT for Screening, Colorectal Cancer Screening Committee, World Endoscopy Organization (5), the majority of manufacturers of quantitative FIT systems had accepted the strategy to report data as weight of measurand per weight of faeces. This strategy, although presenting the great advantage of being relatively easy to perform, for both manufacturers and users, does not fully fit one of the starting requirements of quantitative measurements, the need to achieve the required standards in each phases of the analytical process. For both f-Hb and calprotectin examinations, documentation of the weight of collected faeces was supported by manufacturers; however, these data were obtained by "home-made" measurements, without reference methods or standard protocols, and without providing information on the imprecision of the estimates. Thus, the reported information can be considered as "nominal expected values", of little value in quantitative measurements (6,7). These values widely used both in the literature and by manufacturers following the suggestions of expert working group (EWG) on FIT for Screening (5), should be refined introducing standard protocols for faecal sample collection and measurement. Manufacturers should consider implementing these before introducing new sample collection device to the market. Interestingly, the amount of faeces reported by the manufacturers for the majority of quantitative FIT systems available on the Italian market document the same nominal collected material in the rather different specimen collection devices, although experimental measurements shown significant difference in the mean amount of collected faeces (7).

## Suggestions to address current problems

In our opinion, the major efforts required to correctly address the problem can be avoided using the alternative strategy presented by us as a correction to the previously suggested strategy (8), that is, to report results of examinations on faeces in terms of mass of faeces collected per volume of faeces, that is µg Hb/mL faeces rather than µg Hb/g faeces (8). In fact, the volume of sampled materials is closely related to the volume targeted by the sampling collection devices. This volume, corresponding to the "free volume" of the sampling probe as defined in a previous study (7), should be easily available from production technical data and it is difficult to understand the difficulty of manufacturers in supplying these data (a specific request was made to companies, by us in 2015, but was unfulfilled). The use of the volume of faeces targeted by the sampling devices affects only the reporting of the results of examinations and should be easily included by users.

Two important aspects of the examination process can be solved with this change. Firstly, results are correct from a metrological point of view (since a target volume of faeces is collected without information on the relative density of the collected material). Secondly: unambiguous information can be generated to standardise sample collection device probes and the target volume of faeces that should be collected. Interestingly in the specimen collection devices most widely used in FIT systems, the ratio between the amount of collected faeces (ng) and the sampling volume (mL = $mm^3$) obtained, measuring the volumes by 3D RX investigation, falls close to expected (range, 0.99–1.29 mg/mL).

The major problems arise only from faecal materials collected out of the sample collection component of the probe and should be avoided, perhaps by supporting providing information on improved sampling procedures to patients. The use of the documented amounts of faeces reported by manufacturers introduces a higher overall uncertainty on the result of the examination. The ratio between the mean amount of collected materials and the documented amounts ranged from 71% to 113% with imprecision up to 23% (7).

Another interesting aspect of this change involves the use of FIT for calprotectin measurements presenting unformed (liquid) faeces for long periods. This presents the opportunity to collect the desired amount of liquid samples using pipettes rather than the probes on the specimen collection devices. Usually at the present time, liquid samples are not examined in many laboratories (9). A study to assess the usefulness of this specimen collection technique must be done, in IBD patients, to fully understand the clinical utility of calprotectin before simply not examining this kind of samples. Higher levels of faecal calprotectin should be associated with the magnitude of the inflammatory process, which is related also to the consistency of the faeces materials, and this hypothesis cannot be assessed from currently available data.

## Conclusions

Classification of devices and methods for faecal tests according to the incoming European (EU) *in vitro*

 

diagnostic medical devices regulation (10) is not as yet defined, and also the class of this test is not as yet defined. The introduction of faecal tests based on self-sampling among those methods with full metrological and clinical performances assessed before the introduction on the market is unlikely (III or IV class). Documentation in these more restrictive classes of devices should be justified by the use of test to assess the risk in individuals.

A more detailed use of f-Hb concentrations requires a more accurate investigation of tests before their application in clinical settings. In particular, the evidence of an increased risk of CRC (11) in subjects with f-Hb concentrations between the limits of detection and quantification of quantitative methods requires further attention to the standardisation of many aspects of FIT. Therefore, the presentation of the appropriate Clinical Laboratory Standards Institute (CLSI) protocols to standardise and assure quality and metrology on both pre-analytical and analytical phases of faecal tests (12) should be a main issue for the IFCC Scientific Division Working Group on FIT (13).

The large number of guidelines, consensus papers and investigations of f-Hb cut-offs produced every year by experts on decisional limits and the optimal strategy to use occult blood tests, both qualitative and quantitate FIT represent an evident case of resource-wasting, that should be reduced through improvements based on use of standardised CLSI protocols.

Over recent years, significant efforts have been expended to investigate the clinical significance of an increased f-Hb concentrations and on how to use these data to stratify risk in the screening programmes (11). An important limit of this kind of information arises from the impossibility to transfer data to other settings until performance and commutability of methods are assured by standard protocols.

A further consideration should be addressed to the use of FIT in different health models. Decisional process in reducing the risk of cancer on an individual, may be strongly misguided by data obtained to reduce neoplastic "risk" in a screening population, in particular comparing data obtained from qualitative FIT with laboratory obtained quantitative FIT results, without harmonisation of procedures. So, until now, all the efforts expended are not able to increase the quality of FIT and the overall performance of the screening programmes whether organised, opportunistic, or performed directly by physicians.

After more than 30 years of monitoring of the screening programmes (13), research on decisional f-Hb concentrations and on epidemiological data on sensitivity, specificity and predictive values of FIT do not show significant differences.

The reduction of mortality by screening programmes Is widely confirmed (14) but is time to harmonize and standardize reference on existing methods (15) to reduce the waste of time in work on f-Hb cut-offs and investigate new ways (16) for screening for CRC. Experience in comparing faecal methods, although not yet exhaustive, may represent a useful basis to introduce new methods for faecal DNA (17) or organic volatile compounds (18) measurements on faeces in clinical settings, avoiding the repetition of the errors that have been made over time with FIT.

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