IFCC Scientific Division
Working Group - Faecal Immunochemical Tests
IFCC SD WG-FIT

Reporting of Results - Mass per Volume

Stefano Rapi (AOU Careggi. Florence. Italy).
on the behalf of the SIBioC- GISCoR WG on harmonization of faecal tests.

CRC screening programs in Florence:
FOBT - guiac-based program start in 1982.
From 2002, the program is based on quantitative FIT-Hb

- **with the launch of the screening programs, ongoing investigation of clinical and analytical performance characteristics also began.**

Analytical aspects studied in the investigations:

- ***Method comparisons***
- ***Hb stability in different buffers***
- ***Pre-analytical variables***
- ***EQA (in collaboration with CRR Tuscany Region)***
- ***ISO 15189 implementation in screening laboratories***

Reporting FIT Results:

'Traditional' method of reporting resultss - ng Hb/mL buffer
.....for many years all decision-making was based using these units.

DOI: 10.1093/jnci/djs190
Advance Access publication on April 2, 2012.

COMMENTARY

A Proposal to Standardize Reporting Units for Fecal Immunochemical Tests for Hemoglobin

Callum G. Fraser, James E. Allison, Stephen P. Halloran, Graeme P. Young; on behalf of the Expert Working Group on Fecal Immunochemical Tests for Hemoglobin, Colorectal Cancer Screening Committee, World Endoscopy Organization

WEO World Endoscopy Organization

In 2012, the WEO, CRCSC, EWG on FIT proposed the use of µg Hb/g faeces to improve the harmonization of data across FIT methods.

A few months after the commentary in the JNCI, in a meething on FIT-Hb methods held in Florence, we had the pleasure to meet Callum Fraser and discuss the EWG proposal.

We were not in full agreement with the suggested strategy.

- ***We don't weight samples:*** *we use manufacturers' data on mass of faeces collected and volume of buffer to calculate results.*
- ***Significant variation was reported for the mass of faeces.***
- ***It is not correct to report data simply from theoretical values*.**

More importantly:

…there seems little supporting information on harmonization of faecal sampling .

Preliminary evaluations

Int J Biol Markers 2014; 00 (00): 000-000
DOI: 10.5301/jbm.5000093

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

Stefano Rapi[1], Tiziana Rubeca[2], Callum G. Fraser[3]

Improving performances of Fecal Immunological Tests

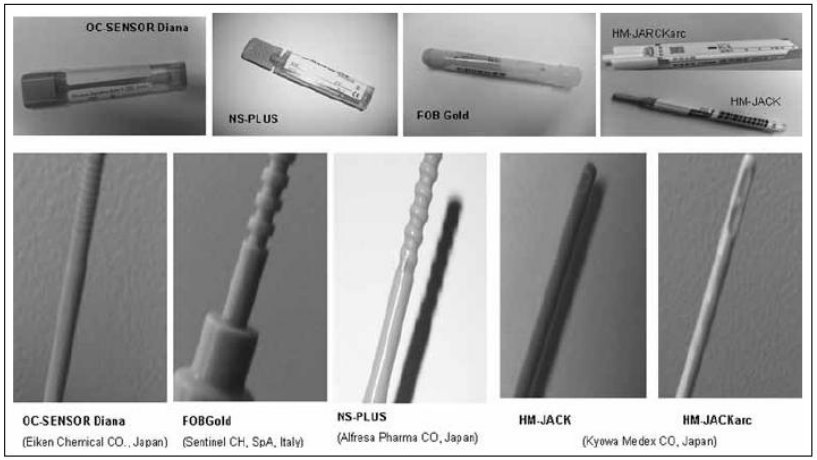


Fig. 1 - *Specimen collection devices and probes/pickes used by different manufacturers for fecal immunochemical test for hemoglobin analytical systems.*

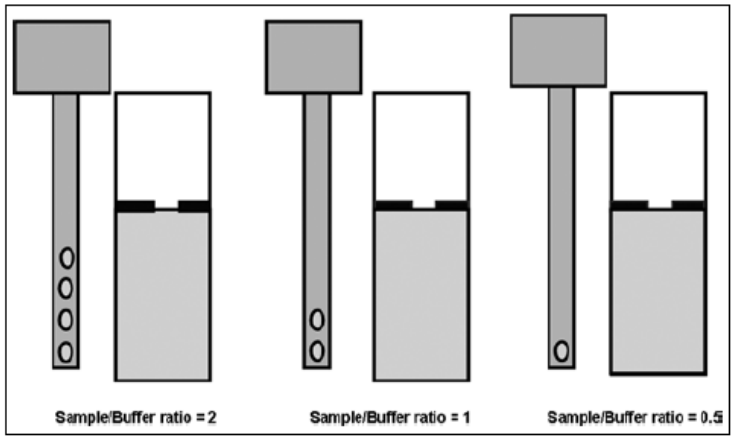


Fig. 2 - *Example of a standardized design for probe/pickers for fecal specimen collection and a suggested relationship between sample size and buffer volume to reduce the overall specimen collection variability.*

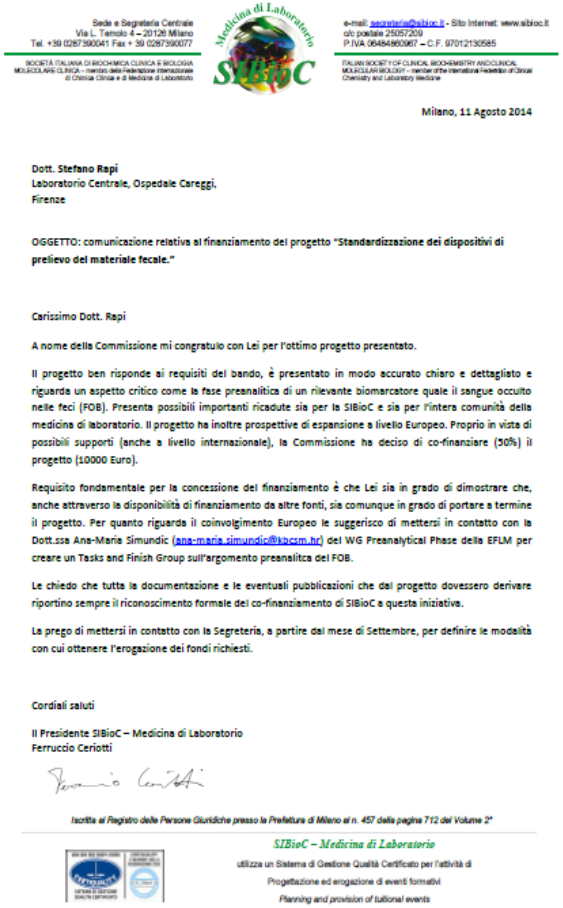
- ***Design of collection devices:***

  different amounts of faeces collected:

- ***Sample device buffers:***

  different amounts of buffer

- ***Different analytical environments***



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SOCIETÀ ITALIANA DI BIOCHIMICA CLINICA E BIOLOGIA MOLECOLARE CLINICA – membro della Federazione Internazionale di Chimica Clinica e di Medicina di Laboratorio

ITALIAN SOCIETY OF CLINICAL BIOCHEMISTRY AND CLINICAL MOLECULAR BIOLOGY – member of the International Federation of Clinical Chemistry and Laboratory Medicine

Milano, 11 Agosto 2014

Dott. Stefano Rapi
Laboratorio Centrale, Ospedale Careggi,
Firenze

OGGETTO: comunicazione relativa al finanziamento del progetto "Standardizzazione dei dispositivi di prelievo del materiale fecale."

Carissimo Dott. Rapi

A nome della Commissione mi congratulo con Lei per l'ottimo progetto presentato.

Il progetto ben risponde ai requisiti del bando, è presentato in modo accurato chiaro e dettagliato e riguarda un aspetto critico come la fase preanalitica di un rilevante biomarcatore quale il sangue occulto nelle feci (FOB). Presenta possibili importanti ricadute sia per la SIBioC e sia per l'intera comunità della medicina di laboratorio. Il progetto ha inoltre prospettive di espansione a livello Europeo. Proprio in vista di possibili supporti (anche a livello internazionale), la Commissione ha deciso di co-finanziare (50%) il progetto (10000 Euro).

Requisito fondamentale per la concessione del finanziamento è che Lei sia in grado di dimostrare che, anche attraverso la disponibilità di finanziamento da altre fonti, sia comunque in grado di portare a termine il progetto. Per quanto riguarda il coinvolgimento Europeo le suggerisco di mettersi in contatto con la Dott.ssa Ana-Maria Simundic (ana-maria.simundic@kbcsm.hr) del WG Preanalytical Phase della EFLM per creare un Tasks and Finish Group sull'argomento preanalitca del FOB.

Le chiedo che tutta la documentazione e le eventuali pubblicazioni che dal progetto dovessero derivare riportino sempre il riconoscimento formale del co-finanziamento di SIBioC a questa iniziativa.

La prego di mettersi in contatto con la Segreteria, a partire dal mese di Settembre, per definire le modalità con cui ottenere l'erogazione dei fondi richiesti.

Cordiali saluti

Il Presidente SIBioC – Medicina di Laboratorio
Ferruccio Ceriotti

Iscritta al Registro delle Persone Giuridiche presso la Prefettura di Milano al n. 457 della pagina 712 del Volume 2°

SIBioC – Medicina di Laboratorio
utilizza un Sistema di Gestione Qualità Certificato per l'attività di
Progettazione ed erogazione di eventi formativi
Planning and provision of tuitional events

Our concept was to produce and investigate a sampling device to obtain information on sampling and engineering variation on the amount of faeces collected and assess if it was possible produce a «standard» device.

Valutazioni preliminari per la proposta di un unico dispositivo di campionamento per la ricerca dell'emoglobina su materiale fecale

Stefano Rapi[1], Callum G. Fraser[2], Filippo Cella[3], Margherita Berardi[1], Tiziana Rubeca[3]
[1]Laboratorio Generale, Dipartimento Servizi, Azienda Ospedaliero Universitaria Careggi, Firenze
[2]Center for Research in Cancer Prevention and Screening, University of Dundee Ninewells Hospital and Medical School, Dundee, Scotland
[3]Laboratorio Prevenzione Oncologica, Istituto Studio Prevenzione Oncologica, Firenze

ABSTRACT

Preliminary assessments for a standard sampling device for fecal hemoglobin detection. Sampling of feces is strongly affected by the lack of harmonisation, with differences up to 20 times in the mass collected for immunological tests for fecal hemoglobin (Hb) used in colorectal cancer screening. Aim of this study was to acquire information on fecal sampling and on the interaction between feces and analytical methods to obtain a reference design for a sampling dipstick. Bias and imprecision of sample collection dipsticks were estimated using gravimetry. Dissolution times of feces were monitored throughout the study. The effect of increasing amount of feces on Hb concentrations was investigated in saline and buffers of different manufacturers using a single analytical method (OC-Sensor, Eiken Chemical Co.). Fecal mass recovered with different devices ranged from 56 to 121% of declared amount (CV range: 8.6÷31.1%). Time of dissolution up to 2 h was observed when lumps of materials were collected. In saline a rapid decrease of Hb values was observed, which was related to the overall amount of feces. Increased Hb values were observed by adding feces to manufacturers' buffers. Solubilisation time, bias and imprecision of sampling of feces were related to device design. Analytical methods are designed to use specific ratios between feces and buffers. The introduction of a standard dipstick design to reduce preanalytical variability may represent a crucial step for fecal test harmonization.

RINGRAZIAMENTI

Lavoro supportato da SIBioC - Medicina di Laboratorio, Bando progetti scientifici in tema di armonizzazione.

We investigated the amount of faeces collected by differents sampling devices and the analytical effects of the amount of faeces on the reaction media using commercial buffers.

Tabella 1
Accuratezza di campionamento dei dispositivi di prelievo utilizzati nei test immunologici per la determinazione dell'emoglobina fecale (FIT-Hb) commerciali valutati

Dispositivo	Teorico[a] (mg)	Halloran[b] (mg)	GMEC[c] (mg)	Recupero medio (CV)[d]		
				Prova 1	Prova 2	Prova 3
OC-Sensor	10	15	11,2	76% (13,2%)	97% (11,9%)	78% (9,7%)
HM-JACKarc	2	4	1,95	112% (21,3%)	121% (8,6%)	116% (14,7%)
NS-Plus	10	14	9,5	56% (30,4%)	79% (23,4%)	77% (11,7%)
FOB-GOLD	10	16	10,0	72% (31,1%)	87% (25,5%)	92% (14,4)

[a]*Quantità di feci recuperate secondo le dichiarazioni dell'azienda produttrice.*
[b]*Valori riportati nel rif.14.*
[c]*Valutazione del "Guildford Medical Device Evaluation Centre" (GMEC) (13).*
[d]*Prove con materiale fecale diversamente strutturato. I valori riportano il recupero medio percentuale rispetto al valore dichiarato dall'azienda produttrice e il CV di una sequenza di 8 campionamenti sullo stesso materiale.*

Significant differences were found:

- *between nominal and observed amounts of faeces*
- *In the Hb results before and after the addition of faeces in commercial buffers*.

To the FIT manufacturers .

On the behalf of the inter – society working group on pre-analytical on FIT we present to you a requirements of information about your sampling devices.

The same request was presented in august to: Alere, Otsuka Co., limited. Japan: Dia Sorin Inc. USA; Eiken Co Japan (via Medical Systems Italy); Kiowa Medex Co. ltd. Japan; and Sentinel Diagnostics, Italy.

The WG were investigating the amount of faeces collected with the different sampling devices.

We would like to know the theoretical volume of sample collected by your device. Considered volume should correspond to the free volume of sample dipstick used by your company. We would appreciate if you could provide us this information.

The importance of this information was explained in our letter, presented in attack with the documents of the WG.

Thanking in advance for your kind collaboration.

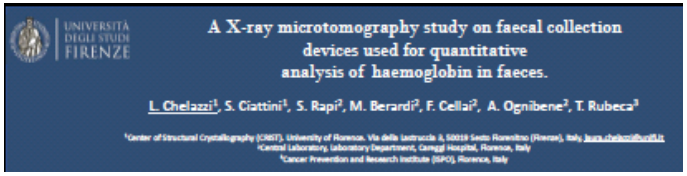
Dr Stefano Rapi

Prof Callum G Fraser

Dr Tiziana Rubeca

… discussing these data with Professor Fraser, we starting to think in detail about the sampling region on the probes of the collection devices…..and we attempted to ask the manufacturer's specifications to verify their data.

Throughout the study, we decided to measure sampling volume of devices using X- ray microtomography.



Interesting information was obtained

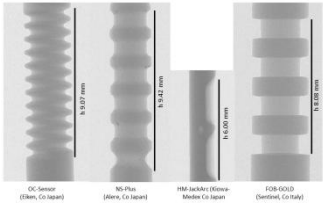
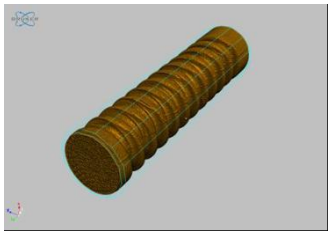


Figure2:Geometric Figure Volume (GFV) obtained calculating the volume of the cylinder that inscribes the probe.



The ratio between mean values of collected faeces and the volume of interest (sampling region) seems to provide fruitful information.

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ORIGINAL RESEARCH ARTICLE

Effects of fecal sampling on preanalytical and analytical phases in quantitative fecal immunochemical tests for hemoglobin

TABLE I - Volumes estimated by geometric calculation of commercial sample collection devices

	GFV (mm^3)	PV (mm^3)	VOI (mm^3)	Collected (mg)	Out of VOI (mg)
OC-Sensor	32.64	24.42	8.22	8.3 ± 0.97	0.43 ± 0.17
NS-Plus	31.89	24.48	7.10	7.08 ± 1.48	0.62 ± 0.36
HM-JackArc	18.24	15.16	3.08	2.38 ± 0.35	0.28 ± 0.21
FOB-Gold	32.3	22.86	9.44	8.36 ± 1.93	0.53 ± 0.29

Recovered (mg/mm^3)	Target (mg)	C/T (%)
0.99	10	83.5
1.0	10	70.8
1.29	2	119
1.13	10	83.6

OXFORD

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First published online October 16, 2015
Correspondence

CORRESPONDENCE

RE: A Proposal to Standardize Reporting Units for Fecal Immunochemical Tests for Hemoglobin

Callum G. Fraser, Stefano Rapi, Tiziana Rubeca

.. proper units must be mass of hemoglobin per **volume** of feces and not mass of hemoglobin per **mass** of feces:

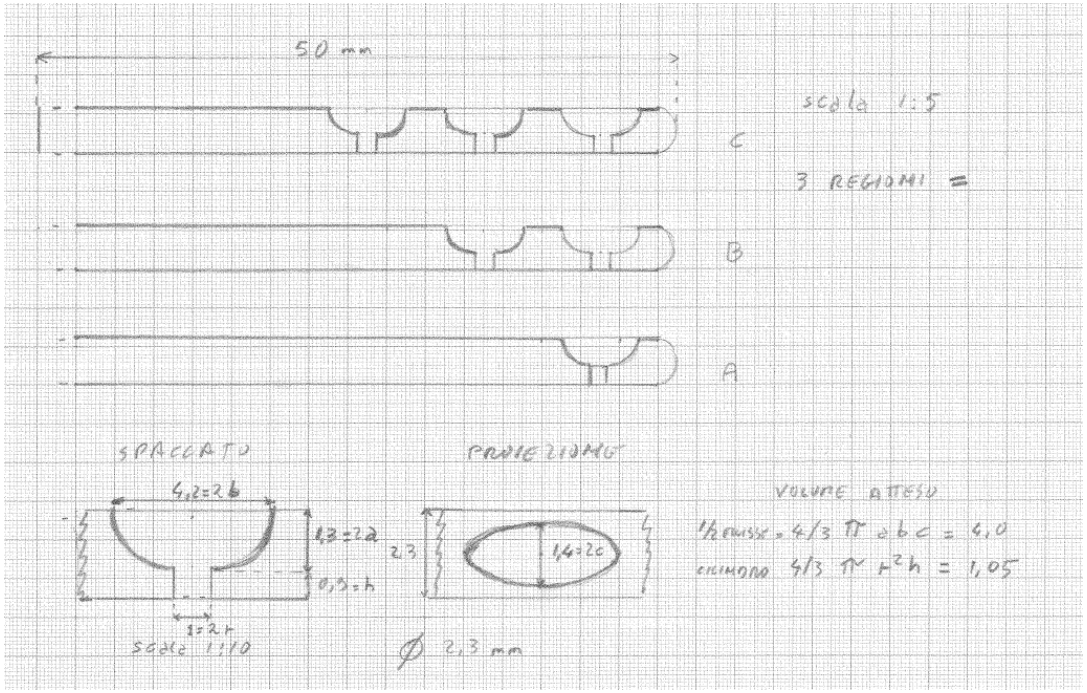
.. ideally, micrograms hemoglobin per milliliter of feces (µg Hb/mL feces), which will give results more accurate than, but similar to, those obtained using the mass of hemoglobin per mass of feces units, since the relative density of feces often approximates to 1.00, and will allow rational reporting of results as integers from zero to over 400.

.. manufacturers of FIT that report results as ng Hb/ml buffer will need to supply detailed and validated information on the volume of feces collected and the volume of buffer in the FIT specimen collection device to allow conversion to the recommended units using the formula:

µg Hb/mL feces = (ng Hb per mL × mL buffer)/(mL feces collected).

… according to the study project, we proceeedd to produce 3 sampling devices using a 3 D printer.

Target: 5 - 10 - 15 mm^3
Ratio 1 : 2 : 3 between sampling volumes.



An error occurred during 3D printing which was communicated by producers.

Volume of the sampling region of the probe with 2 dimples was about 1 mm^3 less than required.

Preliminary data

…to evaluate the performances of our proposed sampling devices, we are using the same strategy used in the earlier paper..

measure the volume of interest (sampling volume) **by X-ray microtomography**

Preliminary data (with SD)

Device	GFV (mm3)	PV(mm3)	VOI(mm3)
1 dimple	24.6±0.2	19.6±0.2	5±0.2
2 dimple	57.5±0.2	48.8±0.2	8.7±0.2
3 dimple	87.7±0.2	72.5±0.2	15.2±0.2

Measure by weighting the mass of collected faeces

Device	nominal	mean (mg)	SD (mg)
1 dimple	5	4.5	0.4
2 dimple	10	8.1	1.1
3 dimple	15	12.5	0.2

Preliminary data

Performances of devices

device	Target (mm3)	VOI (mm3)	Collected (mg)	Recovered (mg/mm3)
1 dimple	5	5	4.5	0.89
2 dimple	10	8.7	8.1	0.93
3 dimple	15	15.2	12.5	0.82

Our design is complex!
The channel on the reverse side of devices
May not be filled with faeces!

Editorial

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

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

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Preliminary considerations:

1- the adoption of correct reporting units fall in laboratory responsibility (15189)

2- in some cases laboratory weight measured samples

Two aspects of the examination process can be solved using mass of hemoglobin per volume of faeces as reporting units in device based methods.

- **Firstly, results are correct from a metrological point of view** (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.**

Introducing a target volume and reference faecal mock materials (known relative density) might harmonize The pre-analytical variation of collection using devices.

A complementary consideration submitted to the WG-FIT

The European legislation on IVD published last year provides for the assignment of tests into different 'risk classes' according to clinical use.

A careful evaluation of the assignment class is necessary for tests such as FIT-Hb

I believe this topic is an interesting challenge for the JRC and for our IFCC SD WG-FIT

Thank you very much for your kind attention.

Acknowledgments: Dr. Tiziana Rubeca
Prof. Callum Fraser
Dr. Filippo Cellai

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