Analytical performance of the novel EQA material for Fecal immunochemical test for hemoglobin

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Introduction

EQA (external quality assurance) program for FIT (fecal immunochemical test for hemoglobin) is performed in many countries, and its demand increases as FIT is widely used in organized colorectal cancer screening programs. The materials used in FIT EQA vary from buffer-based liquid control, and lyophilized stool, to material that mimics stool: artificial stool. The merit to use material that simulates actual sample, stool, is not only to be compliant with ISO15189 but also to suit various methods and sampling devices in FIT. The development of artificial stool material has been awaited by laboratories using FIT to evaluate errors and precisions in specimen collection.

Aims

We assessed the analytical performance of the new artificial stool developed in HECTEF (Health Care Technology Foundation) to evaluate this artificial stool material was suitable as an EQA material for FIT.

Figure 1. New artificial stool and its vial

Methods

1) Sample collection

The material (Figure 1) was collected with the sampling probes of OC-Auto Sampling Bottle 3 (Eiken Chemical) (Figure 2) and the collection amount was calculated by comparing the weight before and after sampling the artificial stool.

2) Uniformity

·The uniformity during production

The material was sampled from 10 different points. Hemoglobin (Hb) concentrations were measured using OC-Sensor DIANA. Evaluation was performed based on "Uniformity of dosage units" section of the pharmacopoeias[1].

·The uniformity between the vials

Three vials were used to sample from three points in each vial. Hb concentrations were measured and coefficient of variation (CV) from the average of Hb concentrations were analyzed.

3) Stability of Hb

The materials stored under different temperature conditions were compared with the material stored frozen at -20 °C (control)

·at 4 °C for 20 months ·at 30 °C for 1 month ·freeze-thawing for 5 times

Evaporation from the material was calculated from the weight difference of the samples (including vials) before and after storage at 30 °C for 1 month and the weight of the material (3 g per vial).

Reference 1 The Japanese Pharmacopoeia 18th edition (2021)

Results

1) Sample collection: The material was collected with sampling probes (Figure 3) and the collection amounts were 105.5 % compared to theoretical amount (Table 1).

Figure 2. The probe of OC-Auto Sampling Bottle 3

Figure 3. Collected sample with sampling probe

sample	vs. theoretical amount
1	105.7%
2	109.5%
3	102.9%
4	106.7%
5	102.9%
mean	105.5%

Table 1. Collection amounts compared to theoretical amount

2) Uniformity: Uniformity of Hb during production was within the acceptance value of <15.0. Uniformity of Hb between vials was within ±10 % CV with 30 µg Hb/g stool sample (Table 2 and 3). With 90 µg Hb/g stool sample similar results were obtained (data not shown).

$\bar{X}$: The mean of the each recovery rate
M : The standard value
When $\bar{X}$ is 98.5 % to 101.5 %, $M = \bar{X}$.
When $\bar{X}$ is less than 98.5 %, M = 98.5 %.
When $\bar{X}$ is greater than 101.5 %, M = 101.5 %.
$AV : M - \bar{X} + 2.4 \times SD$

sample	µg Hb/g stool	recovery rate
1	30.1	100.4%
2	29.8	99.2%
3	29.5	98.3%
4	29.7	98.9%
5	30.1	100.3%
6	29.8	99.4%
7	29.6	98.6%
8	30.4	101.2%
9	29.9	99.8%
10	29.8	99.5%
SD		0.9%
$\bar{X}$		99.6%
M		100.0%
AV		2.2
Acceptability		Acceptable

Table 2. Uniformity of Hb during production

vial No.	sample	µg Hb/g stool	Mean (per vial)	Meanall	SDall	CVall
1	1	31.0	31.0	31.5	1.4	4.3%
	2	30.5				
	3	31.5				
2	1	31.4	30.5			
	2	30.9				
	3	29.1				
3	1	33.2	33.0			
	2	33.3				
	3	32.5				

Table 3. Uniformity of Hb among three vials

3) Stability of Hb: Stabilities of the material were within ±10 % from the frozen material after storage at all temperature conditions (Figure 4 and 5). Evaporation from the material in a vial was below 1 % at 30 °C storage for 1 month (Table 4).

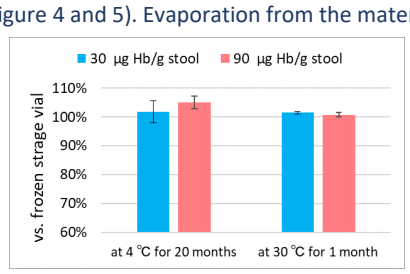

Figure 4. Stability of Hb at refrigeration and 30 °C storage. The error bars show ±SE.

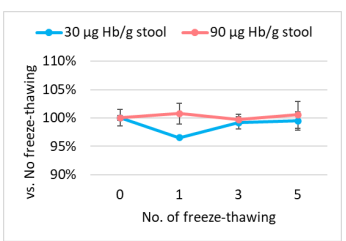

Figure 5. Stability of Hb at freeze-thawing up to 5 times. The error bars show ±SE.

	30 µg Hb/g stool	90 µg Hb/g stool
Initial (g)	10.57981	10.68538
30 °C for 1 month (g)	10.57891	10.68359
Difference (g)	0.00090	0.00179
Evaporaion (%)	0.03000	0.05967

Table 4. Evaporation from the material in a vial

Conclusions

This artificial stool is suitable as an EQA material for FIT because it shows favorable analytical results in sample collection, uniformity, and stability of hemoglobin. The evaluation of this material in the EQA program to show its suitability was performed by Labquality. The result is also reported in a poster at Labquality Days 2024. The use of this material with other antigens such as Fecal Calprotectin is being under development.

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