Implementation of FOB Gold faecal immunochemical testing (FIT) on a Siemens ADVIA Chemistry XPT analyser

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Introduction

Colorectal cancer is the fourth most common cancer and accounts for the second most common cause of cancer deaths in the UK (Cancer Research UK, 2023). The clinical features observed in colorectal cancer are non-specific and include weight loss, abdominal pain, rectal bleeding, iron deficiency anaemia and change in bowel habits (Pin-Vieito et al., 2022; National Institute for Health and Care Excellence, 2023a).

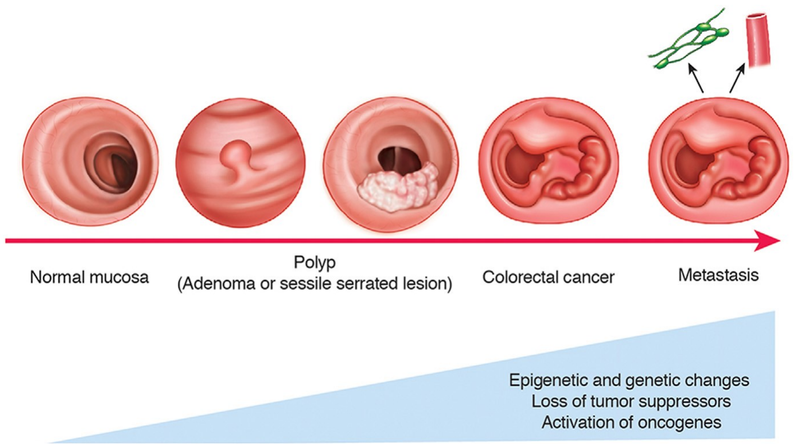

Figure 1: Pathogenesis and nature of colorectal cancer. Most colorectal cancers derive from normal mucosa, leading to the development of polyps, which can develop into colorectal cancer. The gradual development occurs by a range of epigenetic and genetic event, resulting in the silencing of tumour suppressor genes, activation of oncogenes and chromosomal instability (Gupta, 2022:394).

FIT at Stepping Hill

Before June 2023, FIT samples for patients considered as low-risk for colorectal cancer (under NICE NG12/DG30) were originally sent to University Hospital Monklands with an official turnaround time (TAT) of 1 week (average of 7.5 days Monday to Thursday, 7.9 days on Friday from June 2022 for 1 year); with results returned via the post, then by email, both requiring manual data entry at Stockport. FIT samples were sent to Manchester Royal Infirmary (MRI) during the COVID-19 pandemic (from June 2020) to help manage the limited availability of colonoscopy services during and after the pandemic. Patients with faecal haemoglobin ≥10µg/g were offered a colonoscopy. Patients below this cut-off were managed by primary care unless their symptoms persisted (National Institute for Health and Care Excellence, 2017; 2023a).

FIT samples referred to MRI had an official TAT target of 1 week (average of 5.2 days Monday to Thursday, 4.9 days for Friday from August 2021 for 1 year) and were reported via the National Pathology Exchange (NPEX). Both University Hospital Monklands and MRI use HM-JACKarc system for FIT.

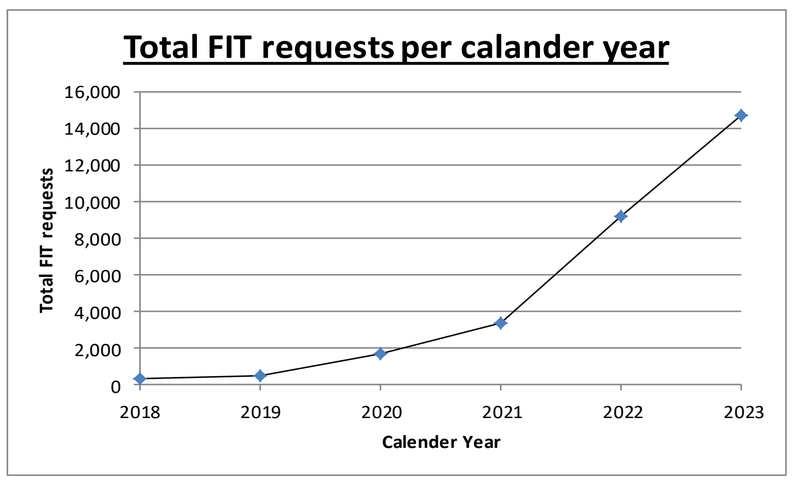

Figure 2: Total number of FIT requests per calendar year at Stepping Hill Hospital. Data shows the increase in demand for FIT from 2020. This increase in demand has made it unfeasible to send samples away to referral laboratories for testing due to logistics, TAT and manual data entry of results.

Aims

To implement FOB Gold FIT assay (from Sentinel Diagnostics, distributed by Sysmex) in-house at Stepping Hill Hospital (SHH) to improve FIT TATs to the next working day. This service will be available for both routine and high-risk patients, allowing clinicians to refer patients in a timely manner when appropriate. This also eliminates laboratory errors that can occur from the complex processes of referring samples.

Objectives

The performance of FOB Gold was assessed against manufacturer's claimed specifications (stated below) in accordance to ISO 15189. The following claims were investigated:

- Assay precision (≤15.3µg/g, CV≤7.0% or ≤±0.85µg/g)
  (>15.3µg/g, CV≤5.0%)
- Limit of blank (0.53µg/g)
- Limit of quantification (2.33µg/g)
- Assay linearity (from 2.33-170µg/g)

Patient comparison was performed with Addenbrookes Hospital, Cambridge who also use FOB Gold on an a Siemens ADVIA 2400. Assay analytical accuracy was assessed using External Quality Assurance (EQA).

Results

Assay precision

	Standard Deviations of haemoglobin (µg/g)									
	Within-run precision					Within-laboratory precision				
	Low IQC	High IQC	Pool 1	Pool 2	Pool 3	Low IQC	High IQC	Pool 1	Pool 2	Pool 3
Mean:	13.20	45.70	5.00	12.10	22.30	13.20	45.70	5.00	12.10	22.30
SD:	0.40	0.47	0.57	0.45	0.52	0.84	1.15	0.62	0.64	0.66
CV:	3.03%	1.03%	11.40%	3.72%	2.33%	6.36%	2.52%	12.40%	5.29%	2.96%

Table 1: Summary of precision data obtained using the EP15-A2 protocol. Internal quality control materials and three pooled patient samples were tested 3 times a day over 5 days. This allowed the estimation of within-run precision and within-laboratory precision. All results were within the manufacturer's acceptance criteria.

Limit of Blank

	Faecal Haemoglobin	
	ng/mL	µg/g
Mean:	6.15	1.05
SD:	2.18	0.37
LoB:	9.74	1.66

Table 2: Summary of data assessing the limit of blank. 20 replicates of sample buffer containing no faecal sample was analysed, allowing the estimation of limit of blank. The value of 1.66µg/g obtained was higher than the quoted limit of blank of 0.53µg/g. The original was obtained in ng/mL but was converted to µg/g by multiplying the conversion factor of 0.17.

Lower Limit of Quantification

	Spike value of haemoglobin (µg/g)			
	1	2	3	4
Mean:	2.4	2.2	2.4	3.6
SD:	2.61	0.84	0.55	0.55
CV:	108.75%	38.18%	22.92%	15.28%

Table 3: Data obtained for assessing lower limit of quantification. Calibrator diluted to assess lower limit of quantification. Each sample was tested with 5 replicates to obtain a mean, standard deviation and coefficient of variance. The lower limit of quantification was 4µg/g, as the lowest concentration to achieve a CV ≤20%, exceeding 2.33g/g, stated by the manufacturer.

External Quality Assurance

Distrubution	Target value (µg/g)	SHH Result (µg/g)	ALTM (µg/g)	ALTM SD (µg/g)	MLTM (µg/g)	MLTM SD (µg/g)	Z Score ALTM	Z Score MLTM	% from target
173A	5.8	3	6.5	4.18	3.3	N/A	-0.85	N/A	-50.17%
173B	18.4	8	17.0	7.32	12.2	6.13	-1.27	-0.74	-58.42%
173C	35.8	23	31.7	15.09	20.9	8.47	-0.55	0.28	-34.94%
174A	24.5	14	23.1	10.31	13.7	4.35	-0.93	-0.03	-44.49%
174B	12.2	3	11.8	5.88	7.5	1.70	-1.51	-2.68	-76.31%
174C	400	265	341.1	166.74	147.3	N/A	-0.46	N/A	-33.87%
177A	0	1	0.6	1.00	N/A	N/A	0.64	N/A	N/A
177B	80.5	42	87.8	32.52	54.6	25.03	-1.42	-0.52	-48.26%
177C	120	71	128.9	57.55	72.0	56.60	-1.00	-0.01	-40.64%

Table 4: Results from UK NEQAS EQA samples. Samples provided from UK NEQAS were tested on the Siemens ADVIA Chemistry XPT using FOB Gold. The results were compared to the All Laboratory Trimmed Mean (ALTM), Method Laboratory Trimmed Mean (MLTM) and the assigned target value. One result (174B) exceeded a Z score of ±2 when assessed against MLTM (at -2.68). All other results were within acceptable limits (8 out of 9).

Patient Comparison

Correlation of FIT result between Addenbrooke's and SHH

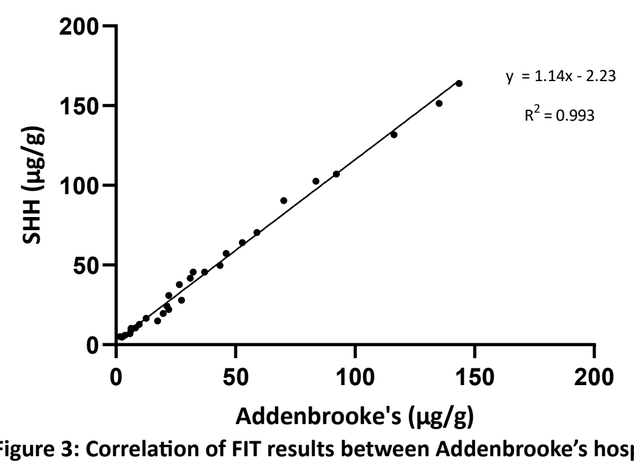

Figure 3: Correlation of FIT results between Addenbrooke's hospital and SHH. Shows the correlation of faecal haemoglobin results between Addenbrooke's hospital and SHH, indicating significant correlation between methods. Spearman's Rank correlation was significant (P<0.001, r=1.00).

Bland-Altman of FIT between Addenbrooke's and SHH

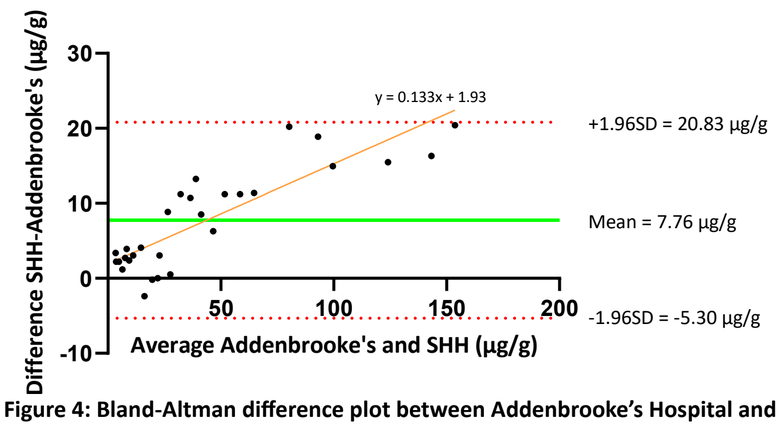

Figure 4: Bland-Altman difference plot between Addenbrooke's Hospital and SHH FIT results. A total of 29 patient samples providing results within analytical ranges were compared between both sites. A concentration dependent bias was observed in SHH results relative to Addenbrooke's Hospital.

Impact on Turnaround Times

After the introduction of in-house testing, the average TAT for samples received between Monday to Thursday was 17 hours or 0.7 days (reduced from 5.2/7.5 days for urgent and routine respectively). The average TAT for samples received on Friday was 59 hours or 2.5 days (reduced from 4.9/7.9 days for urgent and routine respectively).

Discussion

Overall, the assay performed within the manufacturer's specifications in most parameters. The lower limit of quantification was higher than expected (4µg/g rather than 2.33µg/g), however the assay is appropriate for clinical use.

The comparison of patient samples between Addenbrooke's and SHH shown a strong correlation and good agreement, but were significantly different when assessed using a Wilcoxon Signed-rank test (P<0.001). The majority of results (47 out of 50) had the same clinical interpretation. Three patients were considered positive when analysed at SHH although results were close to the clinical cut-off at 10µg/g at Addenbrooke's (ranging from 6-9µg/g). Such differences could be a combination of lot-to-lot variation of reagents and calibrators. There may be differences in performance characteristic between chemistry analysers.

This study did not compare results between different FIT assays. The data from UK NEQAS samples, indicates differences between each methodology likely due to a lack of an international reference preparation; this is being worked on between manufacturers and stakeholders (Benton et al., 2021). A sample analysed using different methods will have different results, however this does not impact on diagnostic accuracy of each assay (Benton et al., 2022).

Using the FOB Gold collection device increased pre-analytical errors leading to the rejection of samples. Rejection rates were approximately 6.9% from the start of 2023 to the end of June 2023 for HM-JACKarc. The rejection rate was as high as 17.7% on the first week of using FOB Gold collection devices. Most rejected samples were due to the loss of extraction buffer; the extraction tubes opened at the wrong end by the patient leading to the loss of buffer. The patient information leaflets were altered and the issue was communicated to primary care. The most recent rejection rates have decreased to 8.5% for March 2024. This rejection rate is comparable to other laboratories in Greater Manchester using other collection devices. We are hopeful to improve this further.

Conclusion

Bringing FIT in-house has improved the TAT to the next working day from one working week when the test was referred externally. Hopefully this will contribute towards improved patient care.

Financially the cost of the in-house service has reduced the cost by 64% when compared to referring the test to a central hub laboratory. FOB Gold can be implemented onto multiple chemistry analysers, allowing a laboratory to implement FIT without additional capital expenditure. This advantage has allowed SHH to implement FIT to meet the growing demand for the test.

Current NICE guidelines [DG56] only recommends the use of HM-JACKarc and OC-Sensor. FOB Gold is therefore not a recommended FIT assay; however NICE recommends further research into its diagnostic accuracy (National Institute for Health and Care Excellence, 2023b). However we know the previous NICE guidelines not so long ago recently suggested using poor "FOB guaiac assays"! There is published evidence that FOB Gold is FIT for purpose.

Performing the assay at SHH will reduced the number of possible "points of failure" where factors within and outside of the laboratory can impact the processing of samples. The direct management of samples and the performance of the assay streamline the processes within the laboratory and reduce both TATs and staff time spent on preparing samples for referral.

Finally, SHH is the only site in Greater Manchester to use FOB Gold assay. This adds resilience to the service of the region as we do not share the same supply chain as other hospitals providing this test.

Acknowledgements

I would like to thank Sue Oddy and David Davy (Addenbrooke's hospital) for arranging the shipments of patient samples to SHH and their technical expertise. This assistance allowed SHH to establish pre-analytical checks of samples with common errors. The information of assay performance at Addenbrooke's helped with the verification of FIT.

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