# Automated immunochemical quantitation of haemoglobin in card collected faeces in screening for colorectal cancer

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## 1. Introduction I

- Screening for colorectal cancer is increasing all over the world, with guaiac faecal occult blood tests (gFOBT) being most frequently used.
- Faecal immunochemical tests (FIT) are now available that allow quantitation of haemoglobin so that the cut-off concentration can be set to give a positivity rate that is manageable in terms of the available colonoscopy resource.
- Many FIT require the collection of faeces into tubes. However, card collection systems are now becoming available.

## 2. Introduction II

- An automated FIT analytical system (analyser plus calibrators, controls and regents) has recently become available and, since it does not require the use of specially designed collection tubes as the sampling devices, is an "open" system.
- The AIMS of the present study were:
  - to investigate the quantitation of haemoglobin in samples collected on cards with this new analytical system and
  - to assess any relationship between faecal haemoglobin concentration and pathology.

## 3. Study population and design

Participants in Phase 1 of the Scottish Bowel Screening Programme who had a positive guaiac FOBT were invited to provide two samples of faeces on a single card collection device (hema- screen DEVEL-A-TAB, supplied by Alpha Labs Ltd, Eastleigh, UK).

## 4. Quantitative analysis of faecal haemoglobin

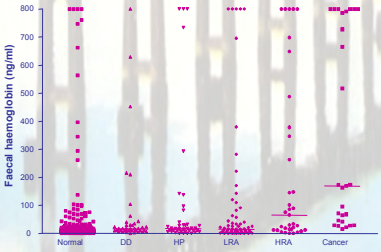
Samples were assayed using immunoturbidimetry on a SENTiFOB analyser using FOB Gold reagents, calibrators and controls (Sentinel Diagnostics SpA, Milan, Italy). Participants were classed according to colonoscopy findings.

## 5. Results I

The 376 participants were classified as follows:

- normal (167 - 44.4%)
- diverticular disease (43 - 11.4%) - DD
- hyperplastic polyps (41 - 10.9%) - HP
- low risk adenoma (63 - 37.7%), that is, 1 or 2 polyps less than 10 mm in diameter - LRA
- higher risk adenoma (35 - 9.3%), that is, 3 or more or any over 10 mm diameter - HRA
- cancer (27 - 7.4%)

## 6. Distributions of haemoglobin concentrations




## 7. Results II

- None of the participants with cancer had haemoglobin < 10 ng/ml but 6 (17.1%) of those with HRA and 106 (33.9%) in the group who do not warrant colonoscopy [normal + DD + HP + LRA] had this finding.
- In contrast, only 18 (5.8%) of those in the group that would not warrant colonoscopy had haemoglobin concentrations > 800 ng/ml whereas 11 (17.7%) of the HRA + cancer group did and 6 (22.2%) of those with cancer.

## 8. Results III

- Those with diverticular disease, hyperplastic polyps and low risk adenoma were not significantly different from the normal group ($P > 0.2$).
- Those with higher risk adenoma had significantly higher concentrations than the groups above ($P < 0.001$) as did those with cancer ($P < 0.001$).
- Those with cancer had significantly higher concentrations than those with higher risk adenoma ($P < 0.001$).

## 9. Results IV

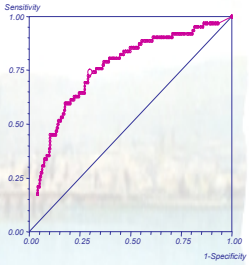
| Colonoscopy Findings | No. | No. (%) < 10 ng/ml | No. (%) > 25 ng/ml | No. (%) > 50 ng/ml | No. (%) > 75 ng/ml | No.(%) > 100 ng/ml | No.(%) > 800 ng/ml |
|---|---|---|---|---|---|---|---|
| Normal | 167 | 63(37.7) | 49(29.3) | 30 (18.0) | 10 (12.0) | 16(6.9) | 4(2.4) |
| Diverticular Disease (DD) | 43 | 10(23.3) | 13(30.2) | 7(16.3 | 6(14.0) | 6(14.0) | 1(2.3) |
| Hyperplastic Polyps (HP) | 40 | 10(25.0) | 15(37.5) | 9(22.5) | 9(22.5) | 7(17.5) | 3(7.5) |
| Low risk adenoma (LRA) | 63 | 23(31.7) | 27(42.9) | 16(25.4) | 14(22.2) | 12(19.0) | 5(7.9) |
| Higher risk adenoma (HRA) | 35 | 6(17.1) | 21(60.0) | 18(51.4) | 17(48.6) | 14(40.0) | 5(14.3) |
| Cancer | 27 | 0(0) | 25(92.6) | 19(70.3) | 16(59.3) | 15(55.6) | 6(22.2) |
| Normal + DD + HP + LRA | 313 | 106(33.9) | 103(32.9) | 63(20.1) | 50(16.0) | 40(13.3) | 18(5.8) |
| Cancer + HRA | 62 | 6(9.7) | 46(74.2) | 37(59.7) | 33(53.2) | 29(46.8) | 11(17.7) |

## 10. Results V

| Cut-off haemoglobin ng/ml | Sensitivity % (95% CI) for cancer | Specificity % (95% CI) for cancer | Sensitivity % (95% CI) for HRA plus cancer | Specificity % (95% CI) for HRA plus cancer |
|---|---|---|---|---|
| 25 | 92.6 (74.2 to 98.7) | 64.4 (59.1 to 69.4) | 74.2 (61.2 to 84.1) | 67.1 (61.5 to 72.2) |
| 50 | 70.4 (49.7 to 85.5) | 85.3 (81.1 to 88.8) | 59.6 (46.5 to 71.7) | 79.9 (74.9 to 84.1) |
| 75 | 59.3 (39.0 to 77.0) | 80.7 (76.1 to 84.7) | 53.2 (40.2 to 65.8) | 84.2 (79.4 to 87.8) |
| 100 | 55.5 (35.6 to 73.9) | 84.8 (80.1 to 88.0) | 46.8 (34.1 to 59.8) | 87.2 (82.9 to 90.6) |

## 11. ROC Analysis

ROC curve generated using (Normal + DD + HP + LRA) as the group classed as without disease and (HRA + cancer) as the group classed as with disease: the area under the ROC curve was 0.7591 (95% CI: 0.6910 to 0.8274) with a haemoglobin concentration of 26.7 ng/ml at optimum sensitivity and specificity.




## 12. Conclusions I

- Haemoglobin concentration can be quantitated in faecal samples collected on simple cards (hema-screen DEVEL-A-TAB) using the SENTiFOB open automated photometric analytical system with FOB Gold immunoturbimetric reagents, calibrators and controls.
- We confirm the very important finding that faecal haemoglobin concentrations rise from normal through HRA through cancer.

## 13. Conclusions II

- No single cut-off concentration gives the ideal clinical characteristics for a screening test, the cut-off concentration used in practice could be selected to give the population positivity rate that could be handled by the available colonoscopy resource.
- In the population studied here, a representative group of gFOBT positive individuals, at 25, 50, 75 and 100 ng/ml haemoglobin, the positivity rates would be 39.6, 26.6, 22.1 and 18.4%.

## 14. Conclusions III

The advantages of the card collection device include:

- simplicity and familiarity for participants,
- ease of posting to and from participants,
- only two samples of faeces on a single card, and
- the ability to use one specimen preparation tube containing buffer for either qualitative or quantitative analysis.

The advantages of the automated FIT used here include:

- the ability to use sample cups or tubes,
- the speed of analysis,
- the analytical reproducibility and,
- most importantly, the ability to vary the exact haemoglobin concentration used to trigger an invitation for colonoscopy on the basis of the resources available.

## 15. Conclusions IV

Here we have examined the role of an automated FIT in a two-tier reflex gFOBT/FIT approach. Undoubtedly the automated immunochemical quantitation of haemoglobin in faeces collected on cards could be used as a first line test in screening for colorectal cancer and further studies are under way to explore this possibility.

***We thank Immunostics Inc for providing the qualitative FIT, Alpha Laboratories Ltd for the loan of pipettes and a centrifuge and Sentinel Diagnostics for the loan of the SENTiFOB analyser and for providing some reagents, calibrators and controls. Professor CG Fraser, through NHS Tayside, held a consultancy contract with Immunostics Inc during the period of this study.***