Validation of a new test in CRC screening – How we did it

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Development of a multi target fecal immunochemical test (mtFIT) for CRC screening

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Current FIT-based CRC screening in the Netherlands

The Fecal Immunochemical Test (FIT) measures 1 marker in stool: hemoglobin

Logistics of the Dutch national CRC screening program
~1.6M participants / year

Adapted from RIVM; Framework for the execution of the dutch colorectal cancer screening program 2021
Current FIT-based CRC screening in the Netherlands

It works:

Stage distribution of screen-detected and symptom-detected CRC’s by subgroups.

Room for improvement:

FIT performance:

- Sensitivity advanced adenoma: ~27%
- Sensitivity CRC: ~79%
- Specificity: ~94%

Esther Toes-Zoutendijk et al. Gut 2018;67:1745-1746
Aim:

Develop a new cost-effective non-invasive screening test suitable for countries with programmatic CRC screening

Recommendations for a Step-Wise Comparative Approach to the Evaluation of New Screening Tests for Colorectal Cancer

**Phase 1:** An initial retrospective evaluation in cancer cases and controls

**Phase 2:** A prospective evaluation of performance across the continuum of neoplastic lesions

**Phase 3:** Demonstration of adequate accuracy in these 2 prescreening phases and programmatic outcomes at 1 screening round on an intention-to-screen basis

**Phase 4:** Comprehensive evaluation of ongoing screening over multiple rounds

Young et al, Cancer, March 2016
Overview new test development

Final goal:
*Validated protein assay for the early detection of advanced neoplasia in stool*
Stool* proteomics-based marker discovery and validation

Sample series 1  
N=22

Sample series 2  
N=291

*whole stool sample

Mass spectrometer

10 proteins

LTQ-FTMS

Q Exactive

Mass & de Wit et al, Annals of Internal Medicine, Nov 2017
Overview new test development

Discovery phase
Mass spectrometry

Protein markers in stool
Whole stool sample

Retrospective validation
Antibody assay

Validation in FIT screening samples (n=1284)
OC-Sensor Sampling device

Preparation of screening trial
Antibody assay

Technology optimization
Stakeholder alignment
Regulatory and QA

Prospective validation
Antibody assay

Prospective screening trial (n=13,300)
FOB-Gold & (OC-Sensor) Sampling device

Final goal:
Validated protein assay for the early detection of advanced neoplasia in stool

Recommendations for a Step-Wise Comparative Approach to the Evaluation of New Screening Tests for Colorectal Cancer
Phase 1: An initial retrospective evaluation in cancer cases and controls

Young et al, Cancer, March 2016
Assay development and analysis

10 Proteins in 1284 FIT samples

1. FIT samples analyzed by Antibody based-assay

2. Whole stool Analyzed by Mass-spectrometry

Bosch & de Wit et al, Annals of Internal Medicine, Nov 2017
De Klaver & Wisse et al, Annals of Internal Medicine, Sep 2021
CART analysis yielded hemoglobin, calprotectin and serpinF2 as best combination = mtFIT

De Klaver & Wisse et al, Annals of Internal Medicine, Sep 2021
mtFIT has a higher sensitivity with equal specificity

3 protein marker panel: hemoglobin, calprotectin & serpinF2

De Klaver & Wisse et al, Annals of Internal Medicine, Sep 2021
The impact of biomarker based screening

Cohort of 20 million people at age 20, followed until age 90 or death, whichever comes first

Current FIT screening:
Biennial age 55-75
11 rounds
73% adherence

Strategies:
• No screening
• FIT screening (73% participation)
• Biomarker based screening (73% participation)

Positive FIT

Diagnostic colonoscopy:
Participation 92%

De Klaver & Wisse et al, Annals of Internal Medicine, Sep 2021
Projected long term benefit of mtFIT for society

Validated mathematical modelling applied mtFIT test parameters to a cohort of 20,000,000 people at age 20, followed until age 90 or death, whichever comes first

Result

↓ Reduction of 12% in CRC incidence*

↓ Reduction of 8% in CRC mortality*

mtFIT cost-effective compared to FIT at a maximum costs per invitee of €59,-

* cross validated

De Klaver & Wisse et al, Annals of Internal Medicine, Sep 2021
### Overview new test development

<table>
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<th>Year</th>
<th>Discovery Phase</th>
<th>Retrospective Validation</th>
<th>Preparation of Screening Trial</th>
<th>Prospective Validation</th>
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<td>Mass spectrometry</td>
<td>Protein markers in stool</td>
<td>Technology optimization</td>
<td>Prospective screening trial (n=13,300)</td>
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<td>Whole stool sample</td>
<td>Stakeholder alignment</td>
<td>FOB-Gold &amp; (OC-Sensor) Sampling device</td>
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<td>2017</td>
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**Final goal:**

*Validated protein assay for the early detection of advanced neoplasia in stool*

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**Recommendations for a Step-Wise Comparative Approach to the Evaluation of New Screening Tests for Colorectal Cancer**

**Phase 2:** A prospective evaluation of performance across the continuum of neoplastic lesions

**Phase 3:** Demonstration of adequate accuracy in these 2 prescreening phases and programmatic outcomes at 1 screening round on an intention-to-screen basis

*Young et al, Cancer, March 2016*
Clinical validation
Prospective mtFIT validation in 13,300 participants of the Dutch national CRC screening program
Preparation of the dataflow between the different parties

1. Invitation Letter + Informed Consent with a barcode
   - Send study package
   - Confirm recipient of IC
   - Signed Informed Consent
   - Declaration of "own risk"

2. Send study package
   - Results letter for both tests

Send study package
- Routine test results (pseudonymized)
- Final result of the biomarkers test
- Clinical Data, including results of routine test, i.e., FIT (quantitative Hb levels), mtFIT (quantitative biomarker levels), colonoscopy, pathology

Biomarkers test results (pseudonymized)
- Biological material – test tubes

Clinical Data, including results of routine test, i.e., FIT (quantitative Hb levels), mtFIT (quantitative biomarker levels), colonoscopy, pathology

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Planning prospective study

- **1-1-2022**: Start study
- **1-3-2022**: Start study pilot
- **1-10-2022**: End of inclusions
- **1-1-2023**: Last colonoscopies performed
- **1-3-2023**: Study complete

**Timeline:***
- **16-11-2021**: Assay verification
- **20-12-2021**: Chain verification
- **7-1-2022**: Start study pilot
- **1-12-2021**: Start study

**Confidential**
Translational research roadmap

- **Protein markers**
  - Marker identification
  - Marker validation

- **Assay development**
  - Robust technology & automation

- **Prospective validation**
  - Prospective stool collections

- **Cost effectiveness**
  - Clinical trial

- **Retrospective stool collections**

- **Dutch screening program from 2014**

- **Test approval & implementation**

**Confidential**
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