

Detector C 2.0:

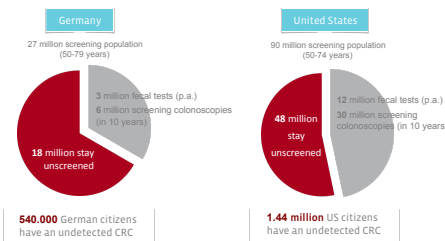
A highly accurate blood-based IVD test for early detection of colorectal cancer with sensitivity and specificity over 90%

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Background:

The EU-5 and US screening population (50-79 yrs) totals 170 million people. Approximately 5.1 million individuals (3%) have an undetected colorectal cancer (CRC). Only 398.000 CRC cases (7.8%) are presently diagnosed per annum (EU-5: 220.000, USA: 176.000). Classical screening methods, including haemocult II (gFOBT) and colonoscopy, detect only 5% of these 398.000 CRC patients. In 4.7 million individuals with CRC the cancer remains undetected. In Germany 73.000 patients per annum are newly diagnosed with CRC. However, only 5.400 patients are diagnosed during participation in screening colonoscopy. Participation in CRC screening using colonoscopy is low (5% – 6% per year) due to the risks involved (bleeding events, colon perforations) and its inconvenience. Participation in CRC screening using gFOBT is decreasing due to the inaccuracy of the test and the difficulty of collecting stool samples.

Unmet Clinical Need in Colorectal Cancer Screening



We previously reported on the discovery and prospective validation of a blood based test (Detector C) for early detection of colorectal cancer (CRC). Detector C measures 202 RNA markers in white blood cells as a response of the host to tumor formation and growth.

Detector C was validated using a prospective, multicenter case-control study with 343 patients, 210 cases with confirmed CRC and 133 controls undergoing a complete screening colonoscopy. Detector C has a validated sensitivity (S') of 90% (95% CI 0.851-0.937) and specificity (S) of 98% (95% CI 0.812-0.930) (Rosenthal A. et al., J Clin Oncol 28:7s, 2010 (suppl; abstr 3580)).

Table 1: Results of Prospective Performance Evaluation of Detector C – Overall and by UICC Stage

	UICC Stage	Frequency	Cumulative Frequency	Fraction	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Sensitivity	All	189	210	0.90	0.851	0.937
	II	52	58	0.90	0.788	0.961
Specificity	All	117	133	0.88	0.812	0.930
	I	48	54	0.89	0.774	0.958
	III	63	70	0.90	0.805	0.959
IV	26	28	0.93	0.765	0.991	

We now present the discovery of Detector C 2.0.

Methods:

From the 462 Affymetrix U133 plus 2.0 expression data used for discovery and validation of Detector C, we selected 445 expression data sets of 291 CRC cases and 154 controls for discovery of Detector C 2.0. Random forest was used for feature (gene) selection and the support vector machine algorithm was employed as classifier in 600 repetitions of double-nested bootstraps to discriminate between cases and controls. Within each repetition, randomly chosen 23 controls and 160 CRC cases served as prospective validation set. The most frequent chosen genes for discrimination between cases and controls formed the consensus signature, namely Detector C 2.0.

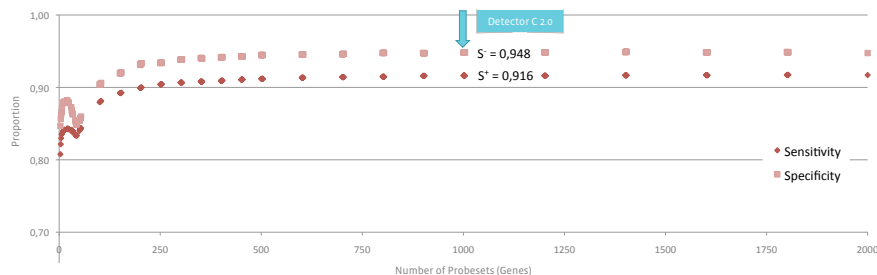
Results:

Second order unbiased estimates for sensitivity and specificity by number of probesets (genes) in the signature are displayed in Figure 1.

Based on a signature consisting of 3 probesets alone, a sensitivity of 0.808 and a specificity of 0.847 can be achieved. With increasing numbers of probesets in the signature, sensitivity as well as specificity first increase, drop, and then increases in a monotonous fashion. However, one must keep in mind, that the expression value of the probesets in the signature are normalized using more than 20000 probesets.

Choosing a signature length of 1000 genes resulted in the following second order unbiased prospective performance estimates: Sensitivity: 0.916 (95% confidence interval (CI): 0.863-0.954) and specificity: 0.948 (95% CI 0.773-0.994). Sensitivity for UICC stage I and UICC stage II cases were 0.93 and 0.94. Sensitivity for high-grade intraepithelial neoplasia was ~0.67, and sensitivity for adenoma ≥ 10 mm was ~0.45.

Figure 1: Second Order Unbiased Estimates for Sensitivity and Specificity by Number of Probesets (Genes) in the Signature



Conclusion:

Using a three times larger discovery set for Detector C 2.0 than for Detector C we improved sensitivity (cancer detection rate) by 1.6% to 91.6%. The most important enhancement is the high specificity of 94.8% of Detector C 2.0. This is six percent higher than the specificity of Detector C, see Table 1.

Even though Detector C has been prospectively validated with very good results, it could not be expected that a larger discovery set leads automatically to a better performance.

Detector C 2.0 is based on a much larger patient set and should be even more robust than Detector C. We will prospectively validate this IVD in the largest case-control study ever performed in early detection of CRC.

Outlook:

Meanwhile we have completed patient recruitment, data entry, and monitoring of study CRC.SCR.4. The expected number of patients are listed in Table 2.

Table 2: Overview of Expected Number of Patients in Study CRC.SCR.4

Patient Group	N
Expected total number of patients	~ 6000
Investigator/clinical sites	53
Patients without any pathological finding during complete colonoscopy	~ 3150
Cancer patients in screening arm	~ 80
Cancer patients in surgery arm	~ 1000
Patients with adenoma ≥10mm	~ 580
Patients with carcinoma in situ and high-grade intraepithelial neoplasia	~ 140

Detector C and Detector C 2.0 will be prospectively validated in multiple (gender, age, performance) matched case-control-studies.

Study CRC.SCR.4 gives also the opportunity to discover and validate additional RNA-based in-vitro diagnostics for large adenoma, carcinoma in situ, and high-grade intraepithelial neoplasia. It might even be possible to discover and validate RNA-based tests for different histologies like villous adenoma, or (sessile) serrated adenoma, or tubular adenoma.

Such additional tests would be performed hierarchically, see Figure 2. The chip platform allows for multiple tests on a single chip.

Figure 2: Hierarchical Sequence of Tests

