

# An Automated Workflow for the Analysis of the <sup>m</sup>SEPT9 Colorectal Cancer Early Detection Marker in Blood Plasma: Epi proColon Early Detection Assay with the InviGenius®



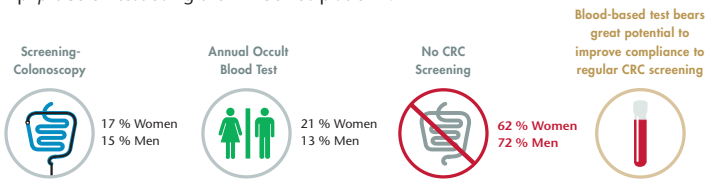
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## Background

Colorectal cancer (CRC) screening has a low compliance rate, although prognosis for CRC patients is more likely to be favorable when disease is detected early. Epi proColon, a blood-based IVD assay available in many countries accepting CE-marking, is a new alternative for CRC early detection and has potential to increase screening compliance. Epi proColon detects bisulfite-converted methylated DNA within the Septin9 gene (<sup>m</sup>SEPT9) in plasma. Assay performance was analyzed in multiple case control studies and prospectively enrolled CRC screening guideline-eligible individuals. These studies demonstrated that detection of <sup>m</sup>SEPT9 in plasma is strongly associated with presence of CRC.

For adoption of <sup>m</sup>SEPT9 testing in clinical routine there is need for automation of the assay procedure. Here we present preliminary data from an automated version of the Epi proColon test using the InviGenius platform.



Source: Zentralinstitut für die kassenärztliche Versorgung, 2009

Figure 1: Accumulated compliance to CRC screening of eligible individuals (55 to 74 years) 2003–2008 in Germany. The additional offer of a blood-based test bears great potential to increase compliance.

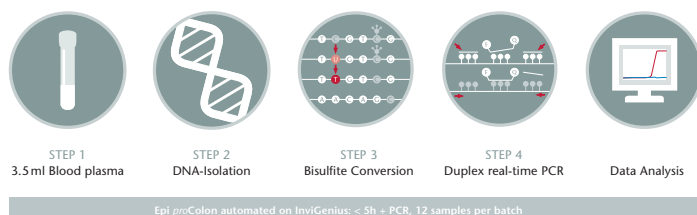
## Methods

Concordance between manual and automated workflows was assessed. Five technical samples (plasma samples spiked with methylated DNA), and ten clinical samples (plasma pools from CRC patients and healthy donors) were processed manually and on the robot.

Manual sample processing was done using Epi proColon Plasma DNA Preparation Kit (M5-01-001) according to the Instructions for Use. Sample processing on the robot was done using new improved chemicals suitable for automation and a modified workflow protocol.



Figure 2: InviGenius liquid handler from STRATEC Molecular



Epi proColon automated on InviGenius: < 5h + PCR, 12 samples per batch

Figure 3: The automated Epi proColon workflow

## Results

<sup>m</sup>SEPT9 sample measurements determined with the automated version were concordant with manual version results. The automated workflow enables standardized processing of 12 samples per batch and up to 24 samples per day. The InviGenius system allows complete traceability of samples, reagents, tips and waste while requiring a minimal footprint (~1m<sup>2</sup>) on the lab bench and minimal manual interaction by the operator.

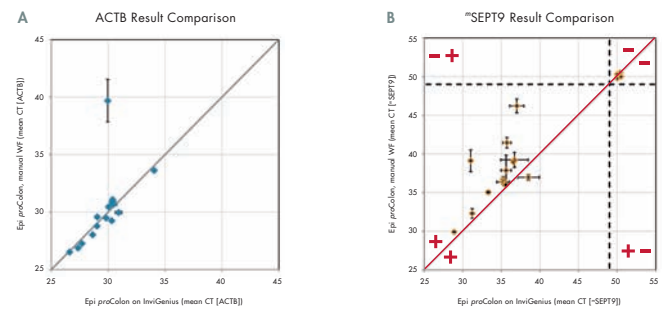


Figure 4: Comparison of automated Epi proColon workflow to manual workflow using Epi proColon: Mean CP values of the manual Epi proColon workflow are plotted against Mean CT values of the automated workflow on the InviGenius. (A): results for  $\beta$ -Actin. (B): results for <sup>m</sup>SEPT9. The manual and automated methods proved to be fully concordant.

Workflow	Sample type	technical samples										clinical samples										controls	
		O	A	B	C	D	E	F	G	H	I	J	K	L	M	N	NC	PC					
automated workflow on InviGenius <sup>1</sup>	result	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	neg	neg	pos				
	Call rate	0/0	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/0	0/0	0/0	3/3				
Epi proColon, manual workflow <sup>2</sup>	result	neg	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos	neg	neg	neg	pos					
	Call rate	0/0	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/0	0/0	0/0	2/2					
Concordance (%)		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100					

<sup>1</sup> samples prepared on the InviGenius were measured in triplicate

<sup>2</sup> samples prepared with Epi proColon CE were measured in duplicate

Figure 5: Concordance between Epi proColon workflow and automated workflow on InviGenius: The detection rate is 100% concordant.

## Conclusions

The liquid handling and magnetic separation capabilities of the InviGenius robot allow the fully automated processing of up to 10 samples and 2 controls in one batch with minor manual interaction. Automation was made possible by optimization of the protocol including the use of new magnetic particles in both the plasma DNA isolation and post-bisulfite conversion purification steps.

Test results for technical samples, clinical samples and controls obtained on the InviGenius are perfectly concordant to test results generated by a diagnostic laboratory using the Epi proColon IVD-CE marked product.

The InviGenius robot provides a cost efficient solution for automated low-throughput processing of plasma samples for the blood based early detection of colon cancer.

