VIII CONGRESSO NAZIONALE GISCoR

WORKSHOP SCREENING CCR REGIONE LAZIO

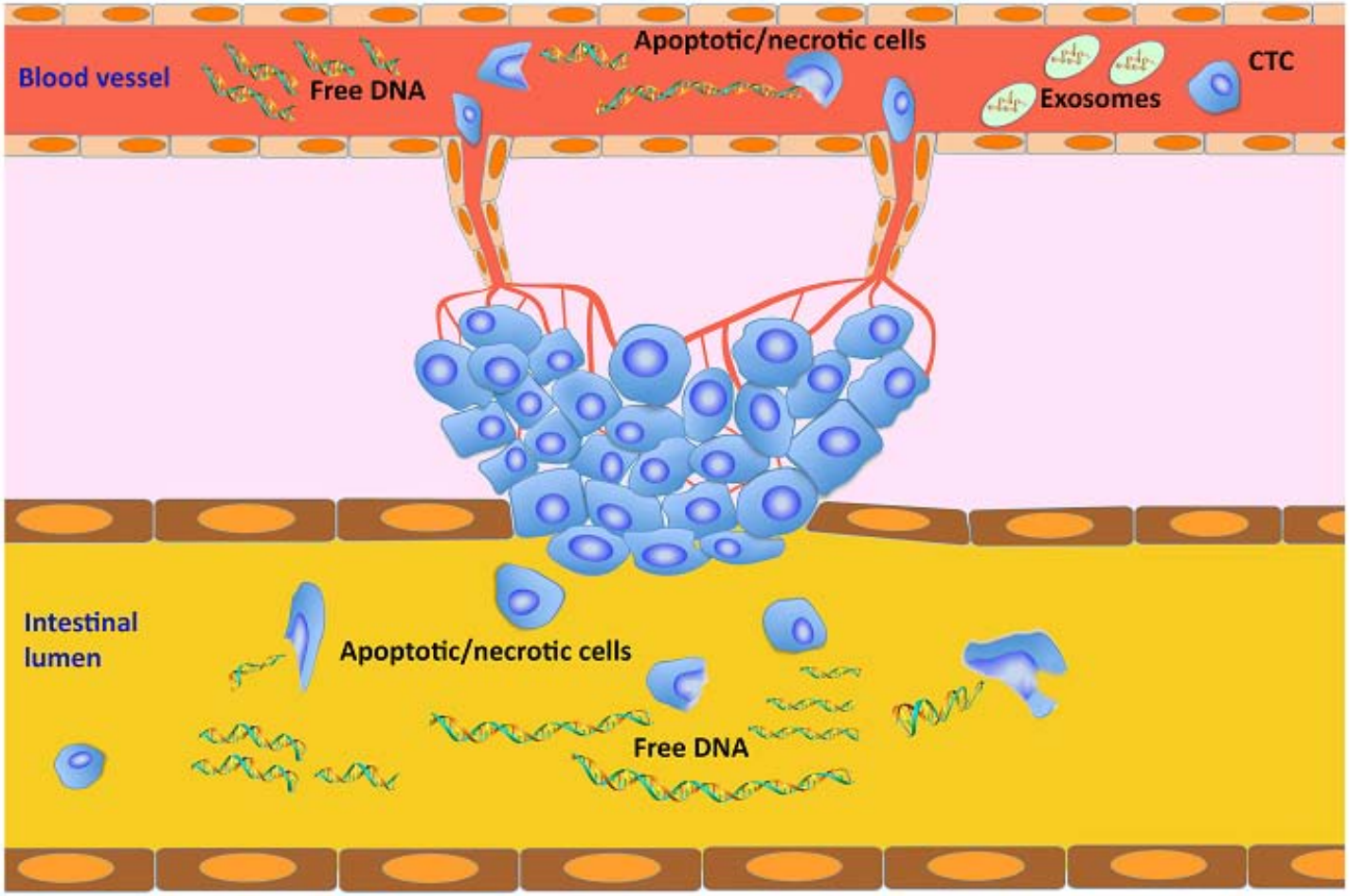
ROMA, 3 E 4 OTTOBRE 2013

Auditorium Antonianum, Viale Manzoni 1

Le possibili applicazioni nello screening delle tecnologie biomolecolari.

Daniele Calistri

I.R.C.C.S.
ISTITUTO SCIENTIFICO ROMAGNOLO PER LO STUDIO E LA CURA DEI TUMORI


Blood vessel
Free DNA
Apoptotic/necrotic cells
Exosomes
CTC
Intestinal lumen
Apoptotic/necrotic cells
Free DNA

Genetic alterations in colorectal cancer

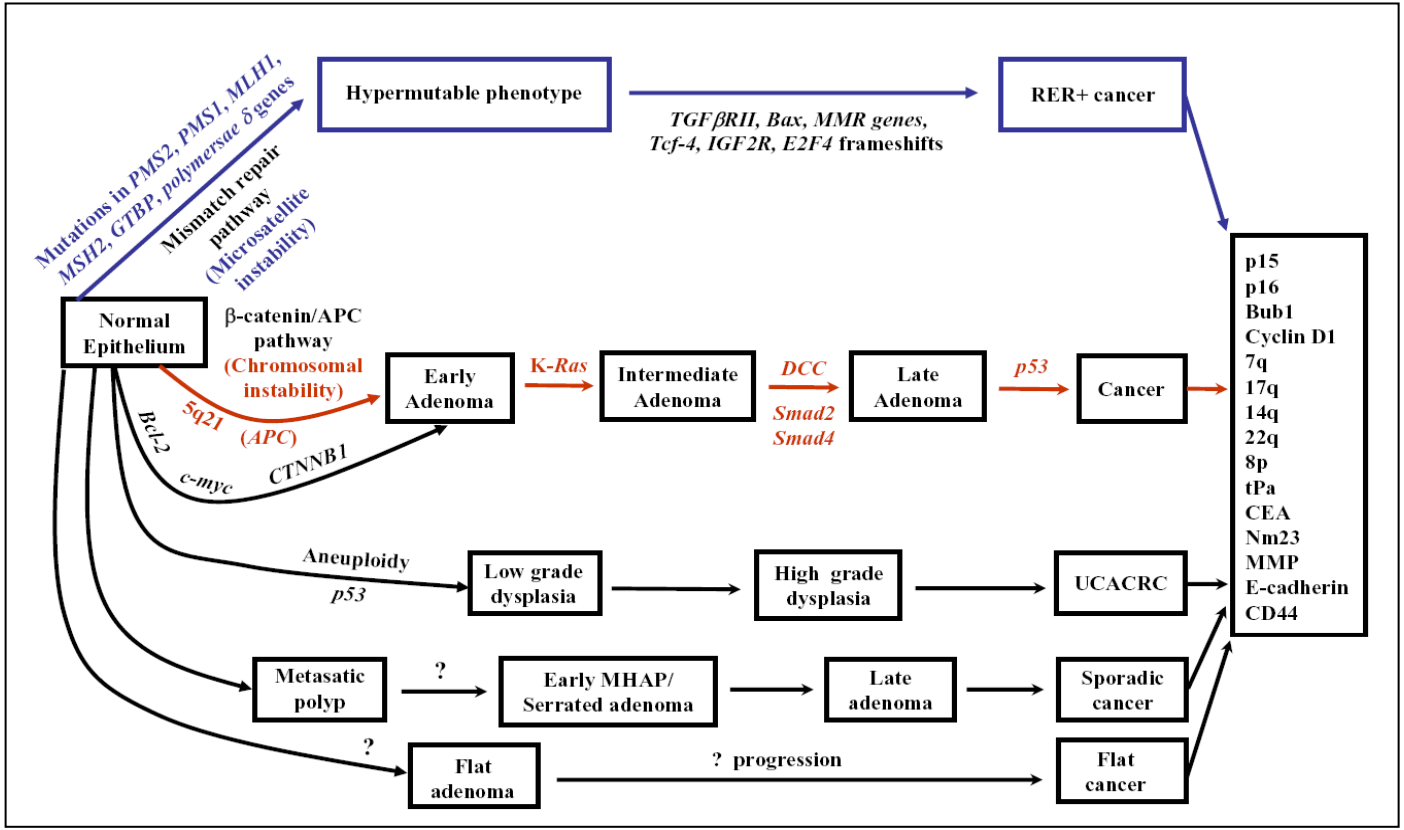


Table 1A DNA Single Markers in Stool

Study	Marker	Test
Puig et al, 2000[176]	*KRAS*	Mut
Traverso et al, 2002a[18]	*APC*	Mutation analysis
Traverso et al, 2002b[177]	MSI	M
Boyton et al, 2003[20]	DIA	Presence of
Wan et al, 2004[178]	*KRAS*	Mutation analysis (all
Müller et al, 2004[35]	*SFRP2*	Meth
Calistri et al, 2004[30]	L-DNA	FL-DN
Lenhard et al, 2005[36]	*HIC1*	Meth
Chen et al, 2005[37]	*Vimentin*	Meth

Table 1B DNA Single Markers in Stool

Study	Marker	Testing Method	Study Population	Sensitivity	Specificity
Huang et al, 2007a[38]	*SFRP2*	Methylation analysis	52 CRC	94%	93%
			10 advanced A	70%	
			11 A	36%	
			8 hyperplastic polyps	38%	
			6 UC	17%	
			24 controls		
Oberwalder et al, 2008[40]	*SFRP2*	Methylation analysis	29 A	21%[b]	100%[b]
			13 hyperplastic polyps	15%[b]	
			26 controls		
Wang et al, 2008[41]	*SFRP2*	Methylation analysis	69 CRC	87%	93%
			34 A > 1 cm	62%	
			26 hyperplastic polyps	42%	
			30 controls		
Calistri et al, 2009[31]	L-DNA	FL-DNA, cut-off 25 ng	100 CRC	79%	89%[c]
			100 controls		
Glöckner et al, 2009[42]	*TFPI2*	Methylation analysis	73 CRC	76%-89%	79%-93%
			75 controls		
Hellebrekers et al, 2009[43]	*GATA4*	Methylation analysis	75 CRC	51%-71%	84%-93%
			75 controls		
Melotte et al, 2009[44]	*NDRG4*	Methylation analysis	75 CRC	53%-61%	93%-100%
			75 controls		
Kim et al, 2009[45]	*OSMR*	Methylation analysis	69 CRC	38%	95%
			81 controls		
Li et al, 2009[46]	*Vimentin*	Methylation analysis	22 CRC	41%	95%
			20 advanced A	45%	
			38 controls		

Table 1C DNA Multiple Markers in Stool

Study	Marker	Testing M
Ahlquist et al, 2000[21]	*KRAS/TP53/APC*	mutation a
	MSI	MSI in BA
	DIA	presence of long DI
Tagore et al, 2003[22]	*KRAS/TP53/APC*	mutation a
	MSI	MSI in BA
	DIA	presence of long DI
Calistri et al, 2003[23]	L-DNA	
	KRAS/TP53/APC	mutation a
	MSI	5-marker
Leung et al, 2004[48]	*ATM/APC/MGMT/hMLH1/HLTF*	methylation
Whitney et al, 2004[26]	*KRAS/TP53/APC*	mutation a
	MSI	MSI in BA
	DIA	presence of long DI
Imperiale et al, 2004[27]	*KRAS/TP53/APC*	mutation a
	MSI	MSI in BA
	DIA	presence of long DI
Petko et al, 2005[49]	*CDKN2A/MGMT/hMLH1*	methylation
Kutzner et al, 2005[168]	*APC*	mutation a
	MSI	MSI in BA
	DIA	presence of long DI
Matsushita et al, 2005[169]	*KRAS/TP53/APC*	mutation a
	MSI	MSI in BA
Itzkowitz et al, 2007[29]	*Vimentin*	methylation
	DIA	presence of long DI
Abbaszadegan et al, 2007[179]	*CDKN2A (p16)*	methylation
	MSI	MSI in BA
	long DNA	presence of long DNA

Table 1D DNA Multiple Markers in Stool

Study	Marker	Testing Method	Study Population	Sensitivity	Specificity
Leung et al, 2007[51]	*ATM/APC/MGMT/hMLH1/HLTF/SFRP2/GSTP1*	methylation analysis	20 CRC	75%	90%
			30 A	68%	
			30 controls		
Huang et al, 2007b[50]	*SFRP2/HPP1/MGMT*	methylation analysis	52 CRC	96%	96%
			10 advanced A	80%	
			11 non-advanced A	64%	
			8 hyperplastic polyps	38%	
			6 UC	17%	
			24 controls		
Onouchi et al, 2008[180]	*KRAS/TP53/APC*	mutation analysis (PCR-SSCP)	33 CRC	55%	89%
			63 controls		
Ahlquist et al, 2008[25]	*KRAS/TP53/APC*	mutation analysis	12 CRC	25%	96%
	MSI	MSI in BAT26	135 A > 1 cm	17%	
	DIA	presence of long DNA (4-site DIA)	469 A < 1 cm	4%	
			341 hyperplastic polyps	5%	
			1473 controls		
Ahlquist et al, 2008[25]	*KRAS/APC*	mutation analysis	19 CRC	58%	84%
	Vimentin	methylation analysis	103 A > 1 cm	46%	
			75 controls		
Itzkowitz et al, 2008[54]	*Vimentin*	methylation analysis	42 CRC	86%	73%
	DIA	presence of long DNA (2-site DIA)	241 controls		
Baek et al, 2009[52]	*MGMT/hMLH1/Vimentin*	methylation analysis	60 CRC	75%	87%
			22 advanced A	46%	
			30 non-advanced A	70%	
			37 controls		
Nagasaka et al, 2009[53]	*RASSF1/SFRP2*	methylation analysis	84 CRC	75%	89%
			27 advanced A	44%	
			29 non-advanced A	28%	
			12 hyperplastic polyps	25%	
			4 ischemic colitis	25%	
			2 UC	100%	
			113 controls		

Table 2 RNA Markers in Stool

Study	Marker	Testing Method	Study Population	Sensitivity	Specificity
Single Markers					
Kanaoka et al, 2004[56]	*PTGS2 (COX-2)*	Nested RT-PCR	29 CRC 22 controls	90%	100%
Chien et al, 2007[60]	*KRAS* codon 12 mutant	Nested RT-PCR and RFLP	29 CRC 20 controls	41%	95%
Leung et al, 2007[51]	*PTGS2 (COX-2)*	RT-PCR	20 CRC 30 A 30 controls	50% 4%	93%
Multiple Markers					
Ahmed et al, 2007[63]	*IGF2/FLNA/TGFBI/CKS2/CSE1L/CXCL3/ DPEP1/KLK10/GUCA2B/Il-12*	Quantitative RT-PCR	20 A > 1 cm 10 IBD 20 controls	> 95%[a]	> 95%[a,b]
Koga et al, 2008[62]	*MMP7/MYBL2/PTGS2 (COX-2)*/TP53	Quantitative RT-PCR	166 CRC 134 controls	58%[c]	88%[c]
Takai et al, 2009[61]	*PTGS2 (COX-2)/MMP7*	Nested RT-PCR	62 CRC 29 controls	90%	100%

Clinical Colorectal Cancer, Vol. 10, No. 1, 8-23, 2011

Table 3A Protein Single Markers in Stool, Other Than Hemog

Study	Marker	Testi
Kronborg et al, 2000[181]	Calprotectin	Immunoassay,
Johne et al, 2001[182]	Calprotectin	Immunoassay,
Tibble et al, 2001[183]		
Kristinsson et al, 2001[184]		
Davies et al, 2002[83]		
Pant and McCracken, 200		
Kim et al, 2003[67]		
Limburg et al, 2003[186]		
Mizuno et al, 2003[187]		
Hoff et al, 2004[188]		
Hardt et al, 2004[189]	Tumor M2-PK	Immunoass

Table 3B Protein Single Markers in Stool, Other Than Hemoglobin

Study	Marker	Testing Method	Study Population	Sensitivity	Specificity
Yuan et al, 2006[190]	Adnab-9	Immunoassay, ODR ≥ 0.05	105 CRC	59%	90%[a]
			29 A	83%	
			27 IBD	33%	
			8 hyperplastic polyps	0%	
			80 controls		
			36 CRC	50%	
			75 controls		
Pucci et al, 2009[77]	Clusterin	Dot blot immunodosage, cut-off level 34.6 µg/g	63 CRC	67%	84%
			50 controls		

Table 3C Protein Multiple Markers in Stool, Other Than Hemoglobin

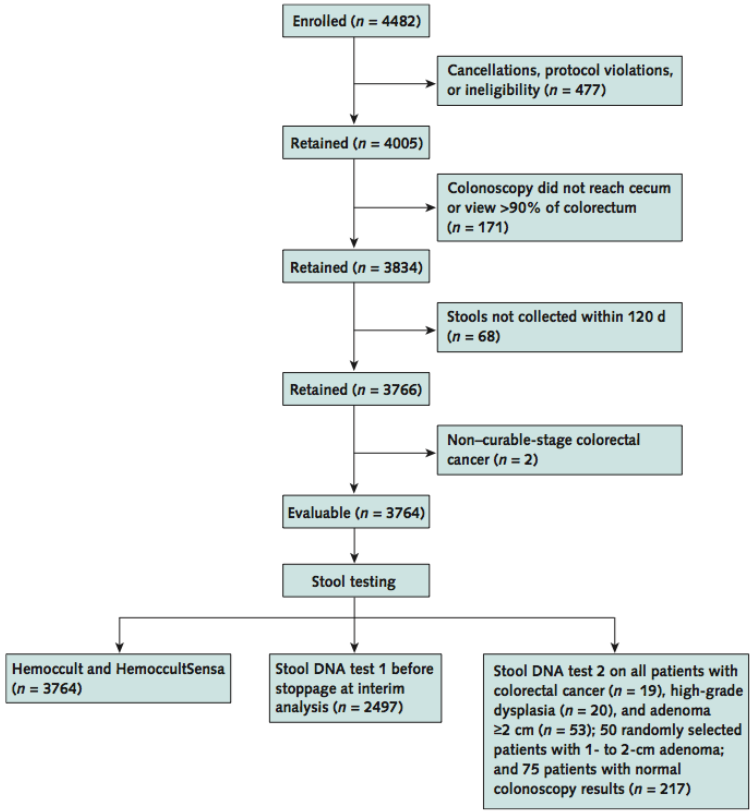
Study	Marker	Testing Method	Study Population	Sensitivity	Specificity
Zou et al, 2007[76]	HNP1-3	Immunoassay, cut-off not reported	20 CRC	35%	90%[a]
			10 A > 1 cm	40%	
			10 upper GI cancer	40%	
			10 IBD	80%	
			30 controls		
Karl et al, 2008[84]	S100A12/hemoglobin-haptoglobin	Immunoassay, cut-off not reported	186 CRC	79% -88%	95%-98%[b]
			113 advanced A	9%-22%	
			252 controls		
Karl et al, 2008[84]	S100A12/hemoglobin-haptoglobin/TIMP-1	Immunoassay, cut-off not reported	186 CRC	82%-88%	95%-98%[b]
			113 advanced A	12%-20%	
			252 controls		

KRAS/TP53/APC	mutation analysis	31 CRC	52%	95%
MSI	MSI in BAT26	403 advanced A	15%	
DIA	presence of long DNA (4-site DIA)	648 polyps	8%	
		1423 controls		

The fecal DNA panel detected 16 of 31 invasive cancers, whereas Hemoccult II identified 4 of 31 (**52% vs. 13% P=0.003**).

The DNA panel detected 29 of 71 invasive cancers plus adenomas with high-grade dysplasia, whereas Hemoccult II identified 10 of 71 (**41% vs. 14% P<0.001**).

NEJM 351:2704-14, 2004



Ann Intern Med. 2008;149:441-450

Table 5. **Presence of DNA Markers in Tumor Tissue***

Marker	SDT-1 Panel						SDT-2 Panel				
	Patients, *n*	*K-ras*, %	*APC*†, %	*p53*, %	BAT-26, %	Full Panel, %	Patients, *n*	*K-ras*, %	*APC*‡, %	Vimentin, %	Full Panel, %
Cancer and high-grade dysplasia	20	45	35	25	0	60	35	51	60	63	94
Adenoma ≥1 cm	48	42	38	6	2	63	99	39	73	63	98
All screen-relevant neoplasms§	68	43	37	12	1	62	134	43	69	63	97

Stool DNA test 1 (SDT1) detected 20% of neoplasms, 11% by Hemoccult, **21% by HemoccultSensa**

Stool DNA test 2 (SDT2) detected 46% of neoplasms, 16% by Hemoccult and 24% by HemoccultSensa.

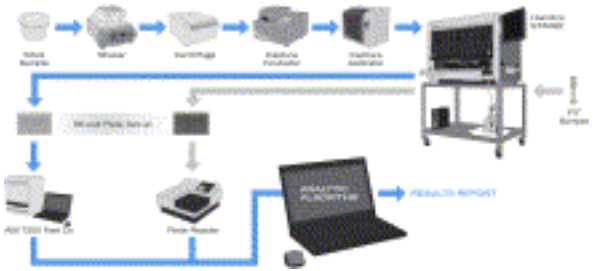
SDT2 detected 46% of adenomas 1 cm or larger, 10% by Hemoccult and 17% by HemoccultSensa.

Problems: SDT-2 specificity was 84%, 96% Hemoccult and 95% HemoccultSensa

Ann Intern Med. 2008;149:441-450

Clinical Performance of an Automated Stool DNA Assay for Detection of Colorectal Neoplasia

Clinical Gastroenterology and Hepatology, *april 2013, in press*



automated multi-target sDNA assay:
β-actin (a marker of total human DNA)
mutant KRAS
aberrantly methylated BMP3 and NDRG4,
Fecal hemoglobin

459 asymptomatic patients before screening or surveillance colonoscopies and **544** referred patients

90% specificity, identified individuals with CRC with 98% sensitivity
advanced precancers (AA and SSA) ≥1 cm was 57%
for >2 cm it was 73%
for >3 cm it was 83%

Cost-effectiveness analysis of colorectal cancer screening with stool DNA testing

Stool DNA testing every 2 years vs colonoscopy every 10 years: **$195**

A similar comparison in the MISCAN and SimCRC models: **$205 - $213**

Gastroenterology. 2004;126:1270-9

$13.000 per life-year gained by stool DNA test screening compared with no screening: **$57 to $70.**

BMC Cancer. 2006;6:136

Stool DNA testing every 3 years (MISCAN and SimCRC models): **$40 to $60**.

Ann Intern Med. 2010;153:368-377

Multiple Detection of Genetic Alterations in Tumors and Stool
Clinical Cancer Research, 2001

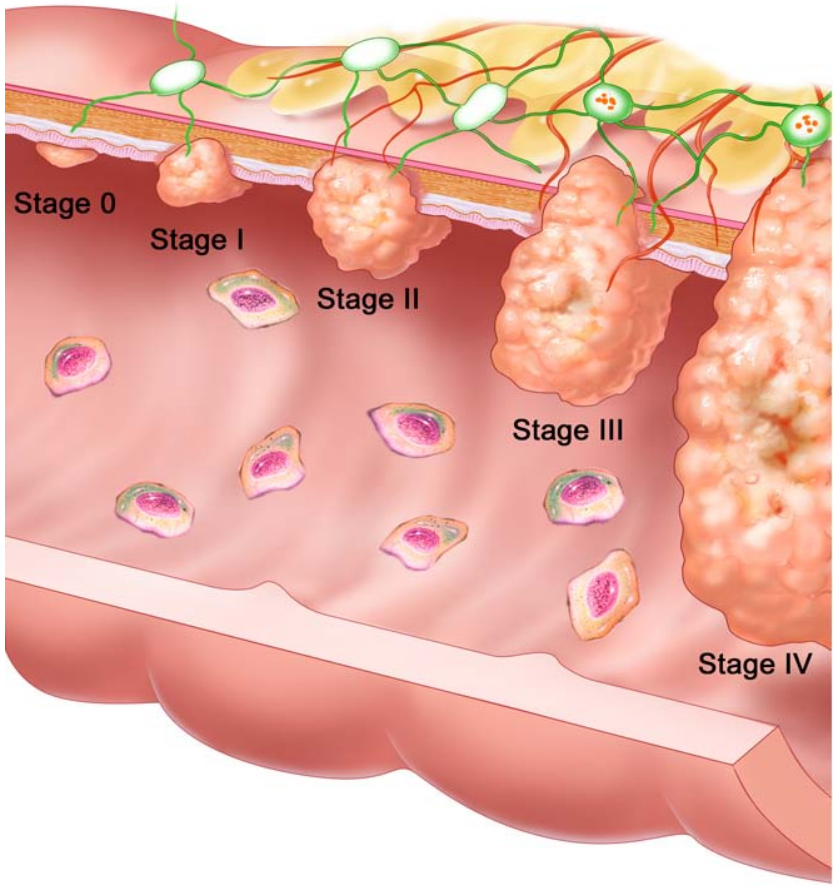
Fecal Multiple Molecular Tests to Detect Colorectal Cancer in Stool
Clinical Gastroenterology and Hepatology, 2003

Detection of Colorectal Cancer by a Quantitative Fluorescence Determination of DNA Amplification in Stool
Neoplasia, 2004

Quantitative fluorescence determination of long-fragment DNA in stool as a marker for the early detection of colorectal cancer
Cellular Oncology, 2009

Fecal DNA for Noninvasive Diagnosis of Colorectal Cancer in Immunochemical Fecal Occult Blood Test–Positive Individuals
Cancer Epid Biom Prev, 2010

- DNA amplification of exfoliated cells in stool has shown to have an important diagnostic potential.



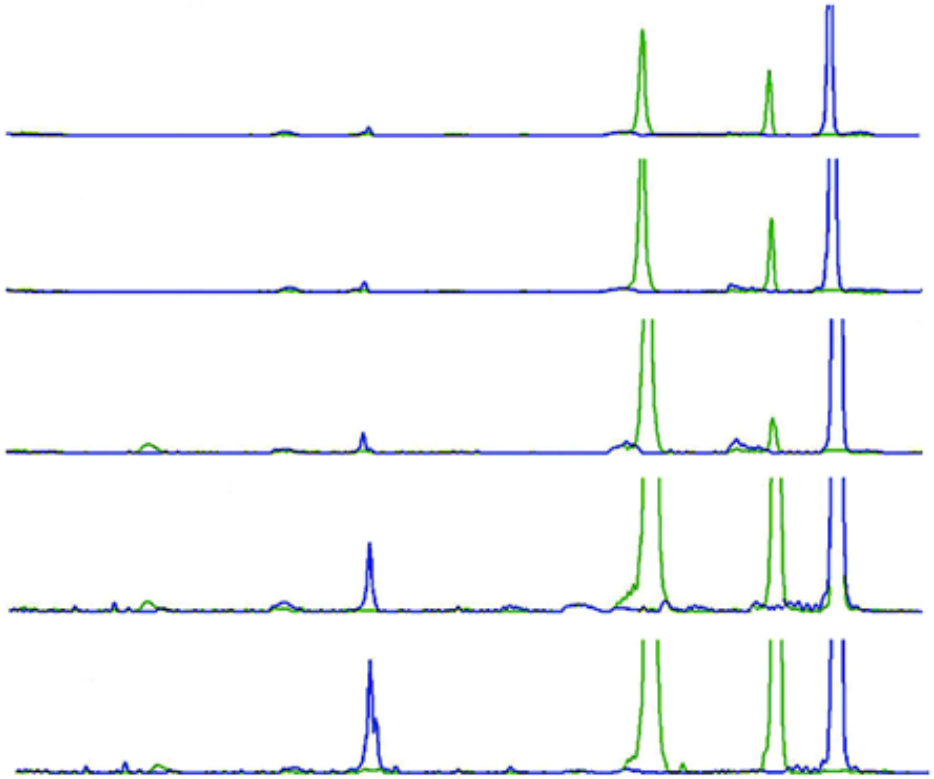
Fluorescent Long DNA (FL-DNA)

DNA extraction from stool

Amplification of different DNA fragment longer than 200 bp

Quantification by fluorescent primers and capillary electrophoresis

Standard curve



European patent
Nord America patent

Sensitivity and specificity of FL-DNA analysis

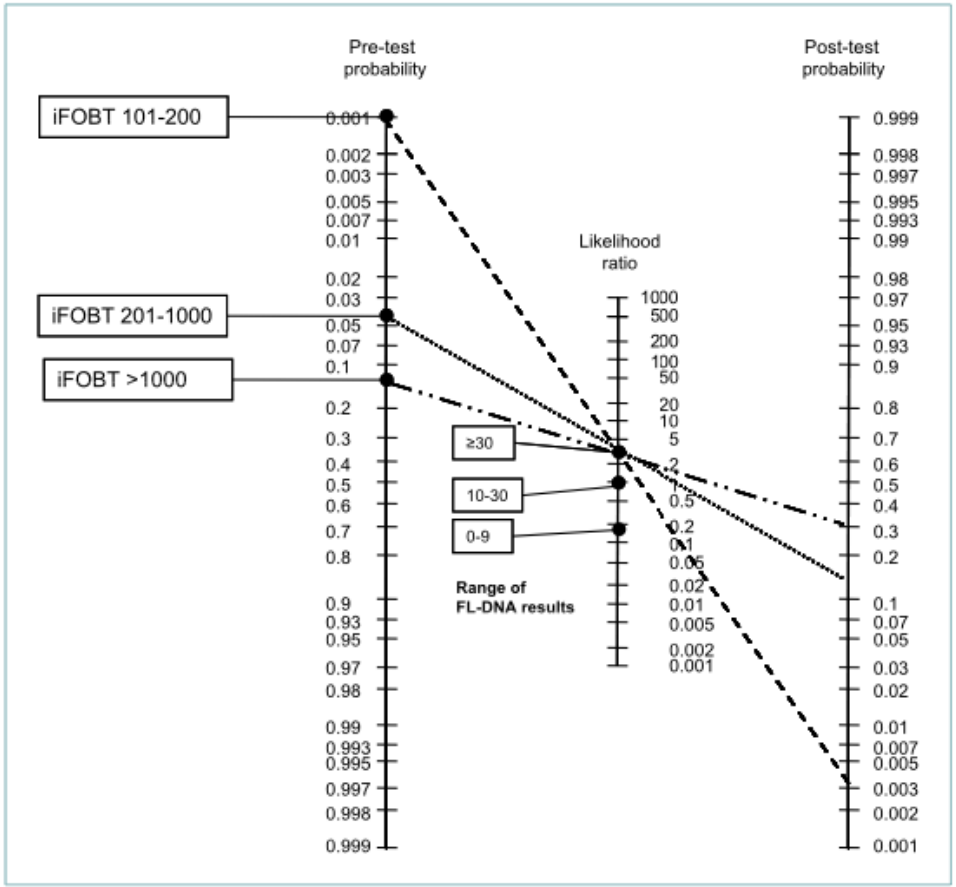
DNA levels	Healthy donors		Patients		% Sensitivity (95% CI)	% Specificity (95% CI)	% Accuracy[1] (95% CI)
Cut-offs (ng)	Positive	Negative	Positive	Negative			
15	*22*	*78*	*84*	*16*	**84** (77-91)	**78** (70-86)	**81** (76-86)
20	*16*	*84*	*82*	*18*	**82** (76-90)	**84** (77-91)	**83** (78-88)
25	*11*	*89*	*79*	*21*	**79** (71-87)	**89** (83-95)	**84** (79-89)
30	*8*	*92*	*70*	*30*	**70** (61-79)	**92** (87-97)	**81** (76-86)
35	*5*	*95*	*68*	*32*	**68** (59-77)	**95** (91-99)	**82** (77-87)
40	*4*	*96*	*65*	*35*	**65** (56-74)	**96** (92-100)	**81** (76-86)

Neoplasia (2004) 6:536–540
Cellular Oncology (2009) 31:11–17

FOBT classes (ng/mL)	*Cases*	FOBT (%)	FL-DNA classes (ng)	*Cases*	FOBT + FL-DNA (%)	Prevalence Cancer (%)
			0-9	*88*	0	0
101-200	*201*	0	10-30	*72*	0	0
			≥ 30	*41*	0	0
			0-9	*102*	0.9	1
201-1000	*239*	4.6	10-30	*92*	4.1	4
			≥ 30	*45*	13.0	13
			0-9	*40*	2.5	2
>1000	*120*	12.5	10-30	*52*	11.3	10
			≥ 30	*28*	30.8	32

560 individuals aged 50 to 69 years with a positive iFOBT were recruited from an Italian FOBT regional screening program

Cancer Epidemiol Biomarkers Prev (2010) 19:2647–54



Case series: 560

Cancer Epidemiol Biomarkers Prev (2010) 19:2647–54

RT FL-DNA

A standardized approach of semi-automatic extraction and DNA integrity analysis for colorectal cancer early diagnosis.

submitted

DNA Extraction

Real Time PCR analysis

RESULTS

Best cut-off value for both FL-DNA analysis approaches

	CE FL-DNA			RT-FL-DNA		
Cut-offs (ng)	% Sensitivity (95% CI)	% Specificity (95% CI)	% Accuracy (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)	% Accuracy (95% CI)
≥5	91 (73-97)	33 (27-39)	38 (32-44)	78 (58-90)	70 (64-76)	71 (65-76)
≥10	91(73-97)	44 (37-50)	48 (42-54)	74 (53-87)	80 (74-85)	79 (74-84)
≥15	78 (58-90)	67 (61-73)	68 (62-74)	70 (49-84)	87 (82-91)	85 (80-89)
≥20	70 (49-84)	79 (73-84)	78 (72-83)	61 (41-78)	91 (87-85)	88 (84-92)
≥25	57 (37-74)	84 (79-89)	82 (76-86)	57 (37-74)	94 (91-97)	91 (87-94)
≥30	52 (33-71)	90 (86-94)	87 (82-90)	57 (37-74)	98 (95-99)	94 (90-96)
≥40	43 (26-63)	96 (93-98)	91 (87-94)	57 (37-74)	99(96-100)	95 (91-97)
≥50	39 (22-59)	99(96-100)	93 (89-96)	48 (29-67)	99(96-100)	94 (90-96)

ADK vs. others **Case series: 241**

MULTICENTRE EVALUATION OF FLUORESCENCE LONG DNA (FL-DNA) METHOD FOR EARLY DIAGNOSIS OF COLORECTAL LESIONS

Case series: 2300

Case series 1:

Subjects of both genders who have consented to take part to the screening program;
Age ≥50 and ≤69 years;
Subject resulted positive to occult blood test (OC-Sensor, Alfa Wassermann);
Subjects candidate to a complete a colonoscopy examination;
Written informed consent.

Case series 2:

Subjects of both genders afferent consecutively to Gastroenterology Units for colonoscopy examinations independently to symptoms or specific pathologies;
Subjects who are not part of the case series 1;
Subjects without a previous cancer history;
Written informed consent.

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Conclusions

Nucleic acids extraction from blood and stool is easy to set up and relatively non-invasive, representing a very attractive tool to detect genetic and epigenetic alterations.

A great variability in terms of concentration, sensibility and specificity values underlines the presence of various pre-analytic and analytic factors that could influence an unequivocal diagnostic impact value.

Standardization in sample collection and analysis are needed to permit a good reproducibility.

Analysis of gene alterations are still expensive and time consuming,

DNA integrity analysis could be a good candidate and its potential could further increase due also to its relatively not expensive approaches.

Multicentre studies in large cohort of individuals are fundamental to clarify the role in clinical settings of these molecular markers.