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Non-invasive detection of colorectal cancer – do we still need the guaiac-based fecal occult blood test?[1)]

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Abstract

Given the simplicity of the method and how it can be applied, as well as proof that it lowers the mortality rate, fecal occult blood testing (FOBT) is currently the most commonly used screening method for colorectal cancer (CRC). However, the test suffers from poor sensitivity, particularly with respect to detecting early stages, as well as low acceptance among the population. Preliminary data on detecting calprotectin and tumour-M2-PK in the stool indicated that a better screening performance could be expected. But these tests also suffer from low sensitivity in detecting early stages and from poor specificity, thus limiting the usefulness of the tests as a result of high follow-up costs. Recently developed immunological tests (I-FOBT) demonstrate significantly increased sensitivity and specificity. I-FOBTs use antibodies specific to human hemoglobin and are therefore not affected by diet and drugs, leading to improved patient partipication. At present, I-FOBTs seem to be the most cost-effective approach for non-invasive screening. The detection of tumour-DNA in the stool opens up a new era in early diagnosis of colorectal cancer. Small trials have pointed to a very high sensitivity of these methods: 62–91% for colorectal cancer and between 26% and 73% for adenomas, with a very high level of specificity (93–100%). The major drawback of this type of testing, compared with other screening tests available today, is its high cost.

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The incidence of colorectal cancer (CRC) doubled between 1960 and 1980 [1]. In statistics on cancer-related deaths in Germany, colorectal cancer is the second most common cause, right after bronchial cancer but still ahead of breast cancer. Each year Germany sees more than 70,000 new cases, with almost 35,000 people dying from this type of cancer each year. The lifetime risk in Germany is 4–6%; from age 50, incidence and mortality rates double with each life decade. Given the long-term process of cancerous degradation, it is possible to prevent cancer through adequate screening and polypectomy or, at least, to detect the tumour in an early stage so as to allow for effective countermeasures to be taken.

Total colonoscopy is seen as the diagnostic "gold standard" around the world when it comes to the early detection of colorectal cancer, as this allows for the simultaneous removal of potentially malignant preliminary stages of cancer. However, this method is still met with limited acceptance among patients, due to, most likely, the preparatory laxative measures as well as physical stress and sedation [2].

The alternative is fecal occult blood testing (FOBT), which Germany added to its early detection programme for cancer in 1977. The benefit of FOBT has been proved repeatedly in a variety of large-scale multicentre randomised studies. Still, due to its poor sensitivity, this test is of limited use. As a result, other non-invasive tests have been developed since the mid-1990s, based on different approaches. Apart from immunology-based FOBTs, these include, for example, the detection of additional blood components, such as leucocyte proteins (calprotectin, lactoferrin), albumin, acute-phase proteins (α1-antitrypsin), tumour-specific metabolic proteins (pyruvate kinase M2) as well as various proto-oncogenes and oncogenes. The following article attempts to provide a critical analysis of the current situation of the most important new fecal tests based on the literature.

Fecal occult blood test

Fecal testing for occult blood is based on the observation that colorectal carcinomas are more likely to bleed than

normal intestinal mucosa. Since many carcinomas bleed intermittently, repeated testing can result in a more reliable detection [3, 4]. A positive FOBT should not be checked; instead, it calls for an examination of the entire colon by means of a colonoscopy.

Guaiac tests

The most common tests, such as the hemoccult test, use filter papers treated with guaiacum resin. The pseudoperoxidase activity of any hemoglobin in the stool sample makes the guaiacum resin turn blue after hydrogen peroxide has been added. The sensitivity in detecting colorectal cancer has been studied in different groups using large populations under screening conditions. For patients with known symptomatic carcinomas, the sensitivity of a single test is over 90% in individual studies [5].

In a prospective study a complete colonoscopy was performed on 3,000 individuals without symptoms and an average age of 63 following an FOBT. The sensitivity of the FOBT in this case was 50% for carcinomas, 12% for all adenomas and 22% for high-risk adenomas (tubular adenomas >1 cm, villous parts or severe dysplasia) [5]. The specificity of the FOBT used was at 94%.

To increase the sensitivity, the test areas were rehydrated for this study prior to its development. However, the increase in sensitivity thus achieved (from 80.8% to 92.2%) was combined with a significantly lower specificity (from 97.7% to 90.4%). As a result, in the US, at least, it is not recommended to rehydrate the test packets [6, 7]. The sensitivity for colorectal cancer is at around 40% with a specificity of between 96% and 98% in a single, non-rehydrated hemoccult test consisting of three test slides [8].

Four large prospective randomised studies have been carried out, whose data on lowering mortality are now available (Table 1). When applied annually, the FOBT can produce a reduction in CRC-associated mortality of 16–33%, since tumours are detected at a stage of a more favourable prognosis. A recent update to the Minnesota study also showed, for the first time, a lower incidence after 18 years of observation. The decrease in incidence amounted to 20% in the annually tested group, as compared to 17% in the biennial tested group [9].

The sensitivity of FOBT for adenomas is significantly lower than for cancer. It correlates with the size of the adenoma and the related increase in the tendency to bleed. In an endoscopically controlled study, sensitivity was merely 24% [5].

Immunological testing to detect occult blood

The benefit of using chemically-based FOBTs is limited by a variety of factors. Apart from moderate sensitivity, as already mentioned, it is frequently dietary factors (e.g., consumption of meat and meat products) that lead to false positive findings [10]. Compliance with certain dietary recommendations, however, reduces the acceptance of the tests [11]. These problems can be avoided by using immunochemical FOBTs (I-FOBTs). These methods were introduced in the early 1990s and detect either hemoglobin or haptoglobin in the stool (Table 2). Another advantage of I-FOBTs is the quantitative analysis of hemoglobin. This allows for normal ranges ("cut-offs") to be adapted to the variable risk profile of individual population groups [12, 13].

A common complaint about larger studies has been the lack of data on reducing mortality. In the meantime, though, a 60% reduction in CRC mortality has been demonstrated for the first time in patients who undergo an annual I-FOBT [14].

Leucocyte markers

Since colorectal neoplasia is characterised by only intermittent bleeding and thus hemoglobin does not make for an ideal marker, leucocyte proteins (calprotectin, lactoferrin) have been proposed as markers since the mid-1990s, because they migrate from the surrounding neoplastic and inflammatory tissue to the intestinal lumen [15]. Calprotectin and lactoferrin are proteins of about 60 kDa and constitute up to 60% of the total protein content of neutrophils. Due to their stability, they are ideal for detecting inflammatory cells in the stool. Its importance in initial and longitudinal diagnoses of inflammatory intestinal diseases has been confirmed repeatedly in numerous studies and is now uncontested [16] (Overview in 17). Especially with a view to increasing sensitivity further, particularly with respect to adenomas, the use of these fecal tests also seemed a reasonable tool for detecting colorectal cancer early. While it is true that the sensitivity levels of 63–90% for carcinomas and 26–80%

Table 1 Overview of randomised studies regarding the use of guaiac hemoccult tests in screening for colorectal cancer.

Author	Participant	Age (years)	Duration (years)	Screening interval (years)	Sensitivity for CRC (%)	PPV for CRC (%)	Reduction in CRC mortality (%)	Compliance (%)
Mandel et al. 1993 [6]	15,570	50–80	13	1	92	2.2	33	75
Kewenter et al. 1994 [36]	33,884	60–64	2	2	81	4.2–5.0	12	63
Hardcastle et al. 1996 [37]	75,253	45–74	7.8	2	64	11 (9.9–11.9)	15	53
Kronborg et al. 1996 [38]	30,967	45–75	10	2	46	10.2–17.7	18	67

CRC: colorectal cancer; PPV: positive predictive value.

Table 2 Overview of studies regarding the use of immunology-based hemoccult tests in screening for colorectal cancer.

Author	Participants (M/F)	Age	Colorectal cancer		Colorectal cancer and adenomas	
			Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Sieg et al. 1999 [39]	621	59 (15–85)	**87** (83–99)	**96**	54	
Nakama et al. 2001 [40]	4270		**89**	**94**		
Greenberg et al. 2000 [41]	554	59.8 ± 11.7	**87.5** (71.3–100%)	**86.2** (83.3–89)	**50.9** (27.7–64.1)	**88** (88.1–99.9)
Wong et al. 2003a [42]	135 (55/80)	58 (38–90)	**89**	**94**	**82**	**94**
Wong et al. 2003b [43]	250 (134/116)	59 (40–81)	**100**	asymptomatic: **87** symptomatic: **99**	**62–65**	**87–93**
Launoy et al. 2005 [44]	7421	50–74	**85** (72–98)	**94** (94–95)		
Morikawa et al. 2005 [45]	21,805	48.3 ± 9.4	**65.8** (55.4–76.3)	**94.3–94.9**	**27.1** (23.9–30.3)	**95.1** (94.8–95.4)
Vilkin et al. 2005 [46]			**100**		**76.5**	**95.3**
Vogel et al. 2005 [47]	116 (44/72)	47	**91** (71–99)	**94** (85–98)		
Hoepffner et al. 2006 [48] (Rapid test/ELISA)	389	59.7	**74.0** (60.4–85)/ **77.7** (64.4–87.9)		**59.7** (47.5–71.1)/ **63.2** (51.7–74.9)	**94.5** (89.9–97.5)/ **96.3** (92.2–98.7)
Li et al. 2006 [49] (Double/triple)	324	53.5 (18–68)	**87.8/95.9**	**96.4/89.2**	**65.1/69.7**	
Smith et al. 2006 [50]	2547		**87.5**	**96.6**	**42.6**	
Levi et al. 2006 [51]	252		**100**	**90**	**74**	**90**
Levi et al. 2007 [12]	1000	63.2 ± 12.1	**94.1** (82–100)	**87.5** (85.4–89.6)	**67** (57.4–76.7)	**91.4** (89.6–93.2)
Shastri et al. 2007 [52]	640 (265/375)	52 (24–88)	**70.9** (51.1–82.4)	**96.3 (94.3–97.8)**	**64.5** (52.7–75.1)	**96.3** (94.3–97.8)

Table 3 Overview of studies regarding the use of leucocyte markers in screening for colorectal cancer (adapted from Haug and Brenner, 2005 [60]).

Author	Marker	Study population (number, age)			Sensitivity (95% CI) (%)		Specificity (95% CI) (%)
		CRC	Adenomas	Control	CRC	Adenomas	
Dubrow et al. 1992 [53]	Lysozyme	n=23, 66 y.	–	n=39, 68 y.	**43** (23–66)	–	**69** (52–83)
Roseth et al. 1993 [15]	Calprotectin	n=40, 68 y.	n=40, 68 y.	n=64, 61 y.	**94** (84–99)	**80** (64–91)	**73** (61–84)
Kronborg et al. 2000 [54]	Calprotectin	n=23, n.a.	n=203, n.a.	n=58, n.a.	**74** (52–90)	**43** (30–50)	**67** (54–79)
Johne et al. 2001 [55]	Calprotectin	n=177, 70 y.	–	n=145, 63 y.	asymptomatic: **64** (44–81) symptomatic: **87** (81–92)	–	**67** (59–74)
Kristinsson et al. 2001 [56]	Calprotectin	n=5, n.a.	n=73, n.a.	n=114, n.a.	**80** (28–99)	**56** (23–66)	**47** (38–57)
Tibble et al. 2001 [57]	Calprotectin	n=62, 68 y.	n=29, n.a.	n=96, 41 y.	**90** (80–96)	**55** (44–74)	**72** (62–81)
Limburg et al. 203 [58]	Calprotectin	n=3, n.a.	n=94, n.a.	n=315, n.a.	–	**37** (28–48)	**63**
Hoff et al. 2004 [59]	Calprotectin	n=12, n.a.	n=787, n.a.	n=1518, n.a.	**63** (35–85)	**26** (2–29)	**76** (74–78)

CRC: colorectal cancer; n.a.: not available.

for adenomas, as identified in eight studies (Table 3), are comparable to those of I-FOBTs, the specificity of 47–76%, however, has been shown to be unacceptable for cost-efficient screening due to the follow-up costs resulting from false positive diagnoses.

M2-PK

The reduced ability to meet energy needs through the glycolytic breakdown of glucose is considered specific to tumour cells and is the result of a dimeric pyruvate kinase (M2-PK) that is increasingly formed during malignant cell transformation. The detection of M2-PK in the stool, therefore, was initially seen as a new tumour-specific marker for malignant processes in the intestinal tract. Retrospective studies, at first for small populations, did identify sensitivity rates of 73% (60–84%), but, similar to calprotectin, this was obtained at the expense of specificity, which was at an unsatisfactory 78% (70–84%); these findings were confirmed in subsequent prospective studies by various working groups with larger patient populations (Table 4) [18]. Own studies [19, 20], as well as those by other authors [21], point to positive test

Table 4 Overview of studies regarding the use of M2PK in screening for colorectal cancer (adapted from Haug and Brenner, 2005 [60]).

Author	Design	Participants (M/F)	Age	Sensitivity (%)		Specificity (%)	
				CRC	Adenoma	CRC	Adenoma
Hardt et al. 2003 [61]	Monocentre, retrospective	78 (58/29)	68.2	**69**	**50**	n.a.	n.a.
Hardt et al. 2004 [18]	Monocentre, retrospective	204 (n.a)	n.a.	**73.8** (60–84)	n.a.	**78** (70–84)	n.a.
Naumann et al. 2004 [62]	Multicentre, prospective	232	n.a.	**85**	**37**	n.a.	n.a.
Vogel et al. 2005 [47]	Multicentre, prospective	138 (61/77)	58	**77**	**48**	**72**	n.a.
Shastri et al. 2006 [19]	Multicentre, prospective	317 (152/165)	56	**81**	**26**	**71**	71
Tonus et al. 2006 [63]	Monocentre, retrospective	96 (54/42)	66	**78**	n.a.	**93**	**93**
Haug et al. 2007 [64]	Monocentre, retrospective	917 (n.a.)	50–70	Colon: **85** (65–96) Rectum: 56 (76–81)	n.a.	n.a.	n.a.
Shastri et al. 2007 [13]	Multicentre, prospective	640 (265/375)	52 (24–88)	**70.9** (57.1–82.4)	**30.4** (19.9–42.7)	**73.8** (69.8–77.6)	n.a.

n.a.: not available.

results in patients suffering from chronic-inflammatory intestinal diseases in relation to inflammatory activity of up to 100%, which disproves the propagated specificity of this test for neoplastic changes beyond any doubt [20, 22].

Molecular markers

The scientific basis for the concept of genetic fecal tests originated with Fearon and Vogelstein, who described molecular changes as an adenoma-to-carcinoma sequence of colorectal cancer at the end of the 1980s [23] (Figure 1). According to this, up to 90% of all carcinomas exhibit mutations in the tumour suppressor gene APC [24], 40–50% mutations in the oncogene K-ras [25] and 50–60% mutations in the p53 tumour suppressor gene [26]. Furthermore, about 50% of all colorectal carcinomas are characterised by the deactivation of a tumour supressor gene on chromosome 18q, which has not yet been identified definitively [27]. Given how common they are, primarily APC, K-ras and p53 were initially seen as promising new tumour markers [28, 29]. The majority of publications, therefore, focused on detecting K-ras mutations at first. The APC gene is almost an ideal candidate gene, as it represents the first

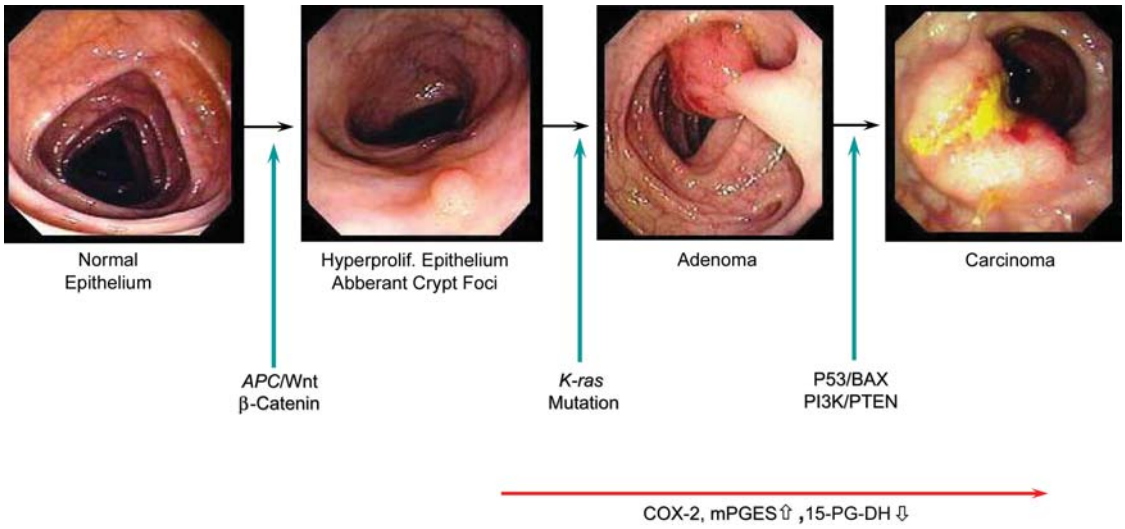

Figure 1 Adenoma-to-carcinoma sequence of colorectal cancer and the mutations occurring in the process.

Table 5 Detection of individual DNA mutations in the stool in patients with colorectal cancer (adapted from Haug and Brenner 2005 [60]).

Author	Marker	Study population (number, age)			Sensitivity (95% CI) (%)		Specificity (95% CI) (%)
		CRC	Adenomas	Check	CRC	Adenomas	
Ratto et al. 1996 [65]	*K-ras*	n=25, 61 y.	–	n=11	**40** (21–61)	–	**100** (72–100)
Villa et al. 1996 [66]	K-ras	n=5, 62 y.	n=42, 59 y.	n=46, 50 y.	**80** (28–99)	**29** (16–45)	**96** (85–99)
Puig et al. 2000 [67]	K-ras	n=11, n.a.	n=22, n.a.	n=30, n.a. 25 pathol. controls	**55** (23–83)	**27** (11–50)	**100** (88–100)
Wan et al. 2004 [68]	K-ras	n=23, 69 y.	n=20, n.a.	n=20, n.a.	**56** (34–77)	**30** (12–54)	**95** (75–100)
Traverso et al. 2002 [30]	APC	n=28, 53 y.	n=18, 63 y.	n=28, 53 y.	**61** (41–79)	**50** (26–74)	**100** (88–100)
Traverso et al. 2002 [31]	BAT26	n=46, n.a	n=69, n.a.	n=19, n.a.	**37** (23–52)	**0**	**100** (82–100)
Müller et al. 2004 [69]	SFRP2 methylation	n=13, 57 y.	–	n=13, 49 y.	**77** (46–95)	–	**77** (46–95)
Loktionov et al. 1998 [70]	SDNAI	n=17, 69 y.	–	n=16, 68 y.	**100** (80–100)	–	**81** (54–96)
Boynton et al. 2003 [71]	DNA integrity	n=27, n.a	–	n=77, n.a.	**56** (35–75)	–	**97** (91–100)

CI: confidence interval; DIA: DNA integrity assay; L-DNA: long-DNA; MSI: microsatellite instability; n.a.: not available.

Table 6 Detection of combined DNA mutations in the stool in patients with colorectal cancer (adapted from Haug and Brenner, 2005 [60]).

Author	Marker	Study population (number, Age)			Sensitivity (95% CI) (%)		Specificity (95% CI) (%)
		CRC	Adenomas	Control	CRC	Adenomas	
Ahlquist et al. 2000 [3]	*K-ras* *P53* *APC* *BAT26* *L-DNA*	n=22, 70 y.	n=11; 73 y.	n=28, 68 y.	**91** (71–99) Independent of stage	**82** (48–98)	**93** (77–99)
Koschiji et al. 2002 [72]	*K-ras* *APC*	n=41, 63 y.	–	n=15, n.a.	**88** (74–96)		**100** (78–100)
Calistri et al. 2003 [73]	*K-ras* *P53* *APC* *MSI* *I-DNA*	n=53, 62 y.	–	n=38, 71 y.	**62** (48–75)		**97** (86–100)
Tagore et al. 2003 [74]	*K-ras* *P53* *APC* *MSI* *DIA*	n=52, 64 y.	n=28, 61 y.	n=212, 63 y.	**64** (49–76)	**57** (36–76)	**96** (93–98)
Leung WK et al. 2007 [75]	*APC* *ATM* *MLH1* *sFRP2* *HLTFMGMT*	n=20, 69 y.	n=30, 70.5 y.	n=30, 70.5 y.	**75** (50.9–91.3)	**68** (46.5–85.1)	**90** (73.5–97.9)

CI: confidence interval; DIA: DNA integrity assay; L-DNA: long-DNA; MSI: microsatellite instability; n.a.: not available.

step in the carcinogenesis of colorectal cancer (gatekeeper function) (Table 5).

When examining the stool for microsatellite instability in the BAT26 marker, microsatellite instability was detected in 37% of 46 proximally localised colorectal carcinomas. But none of the 19 proximally localised adenomas was identified [30, 31]. Such a low level of sensitivity for carcinomas and adenomas, therefore, indicates against the sole use of BAT26 as a molecular marker. But the low sensitivity level is not surprising, because only 15% of all colorectal carcinomas have a microsatellite instability (MSI). An incidence of 25–30% is assumed for proximally localised colorectal carcinomas. Similarly, microsatellite instability in sporadic colorectal carcinomas is often detected only once adenomas progress to carcinomas.

The sensitivity of molecular fecal tests can be increased by *combining different molecular markers* in a marker panel (Table 6). For example, Ahlquist et al. [32] were the first to use a panel of five markers for patients where colonoscopy had confirmed the presence of colorectal cancer and/or adenomas >1 cm, as compared to a control group for whom colonoscopy had yielded no pathological findings. For the study, 15 defined mutations were examined in the genes APC (4 times), p53 (8 times) and K-ras (3 times), as were microsatellite instability (BAT26) and L-DNA (Long-DNA).

Table 7 Comparison of old and new fecal tests in screening for colorectal cancer.

Marker	Colorectal carcinoma (%)		Adenomas (%) Sensitivity	Adenomas + CRC (%)		Costs (€)
	Sensitivity	Specificity		Sensitivity	Specificity	
G-FOBT	26 (13–39)	88–98	12 22	22–34	86–96	0.50–1
I-FOBT	66–100	87–99	20–30	45–80	88–96	Rapid tests 5–8 ELISAs 15–22
Calprotectin	63–90	47–99	26–56 (80)			20–25
M2-PK	69–85	65–78	26–50	55–74	62–78	25–30
DNA-individual	40.56	77–100	27 50	n.a.	n.a.	150–250
DNA-combined	88 (74–96)	90–100	57–82	n.a.	n.a.	300–400

n.a.: not available.

Thanks to the panel used it was possible to diagnose 91% of all colorectal carcinomas and 82% of all adenomas. Specificity was 93% (95% CI 76–99%). When the K-ras marker was excluded, sensitivity was 91% (95%–CI: 71–99%) for colorectal cancer and 73% (95%–CI: 39–94%) for adenomas with a specificity of 100% (95%–CI: 88–100%). The positive predictive value, with K-ras being excluded, was 100%; the negative predictive value was 85% [Overview in 33, 34].

The findings from small patient populations so far have shown a sensitivity of molecular fecal tests of up to 90% in detecting colorectal cancer and, therefore, might be more sensitive and more specific than all other tests available thus far. However, the complicated sample preparation, the costs of staff and equipment and the resulting overall costs of €300–€400 per test are the reasons why this approach is not realistic for colorectal screening at this point [35].

Conclusion

The G-FOBT has been a fixed element in statutory early cancer screening in Germany since the end of the 1970s. Its benefit has been proved repeatedly in a variety of large-scale multicentre randomised studies. Still, due to its poor sensitivity, this test is of limited use.

The development of immunological tests to detect occult stool in the mid-1990s marked an important step towards improving sensitivity, specificity and patient compliance. I-FOBTs have been classified as more cost-efficient tests in the *prevention* of colorectal cancer in Japan and the United States.

By contrast, neither the detection of neutrophil markers, nor of M2-PK in the stool has met initial expectations due to insufficient specificity and the resulting follow-up costs.

Molecular markers are the way of the future. They produce sensitivity rates of 62–91% for colorectal cancer and 26–73% for adenomas, with a specificity of 93–100%. They are limited, though, due to the expenditure in terms of staff and equipment and, thus, the resulting costs (Table 7).

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