

FECAL OCCULT BLOOD TEST (FOBT)

Common Guaiac versus Immunochemical Test



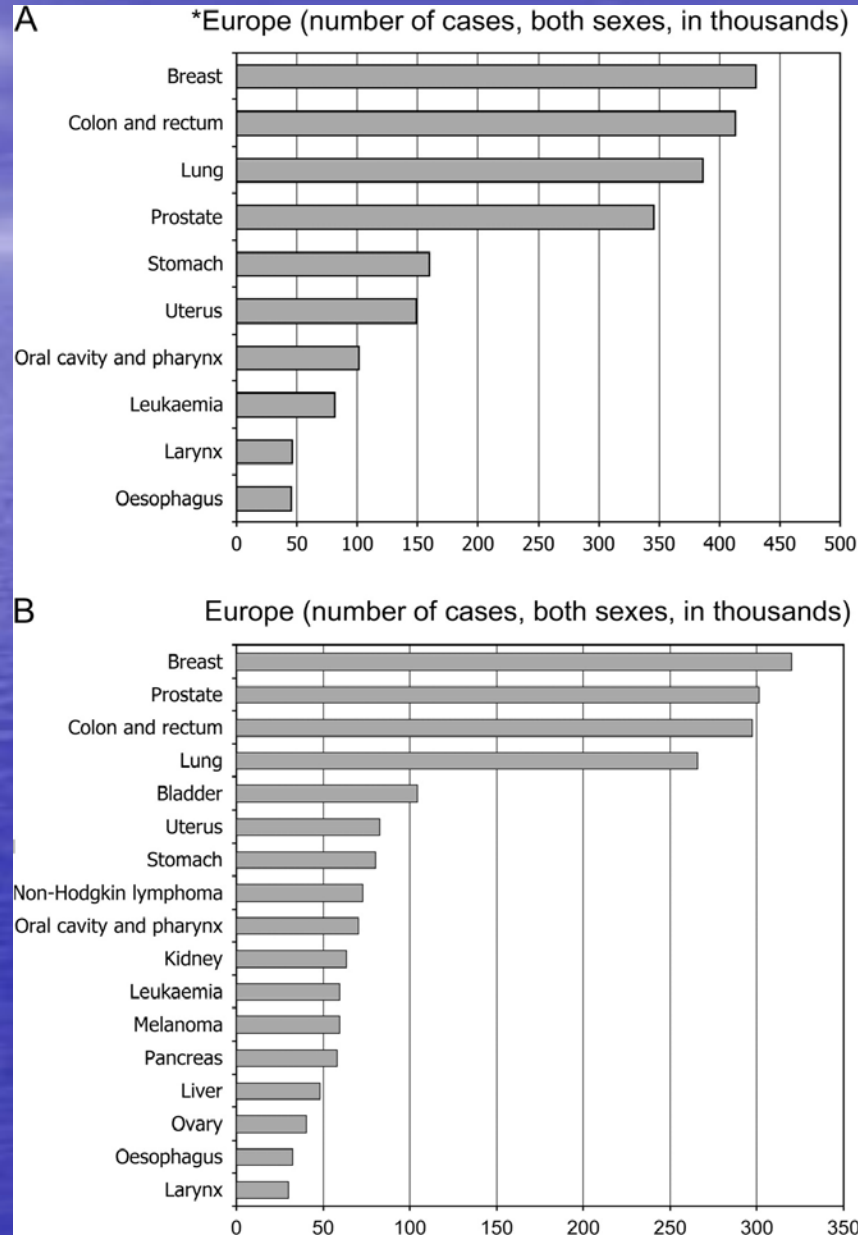
LIMBACH-LABORATORY

HEIDELBERG

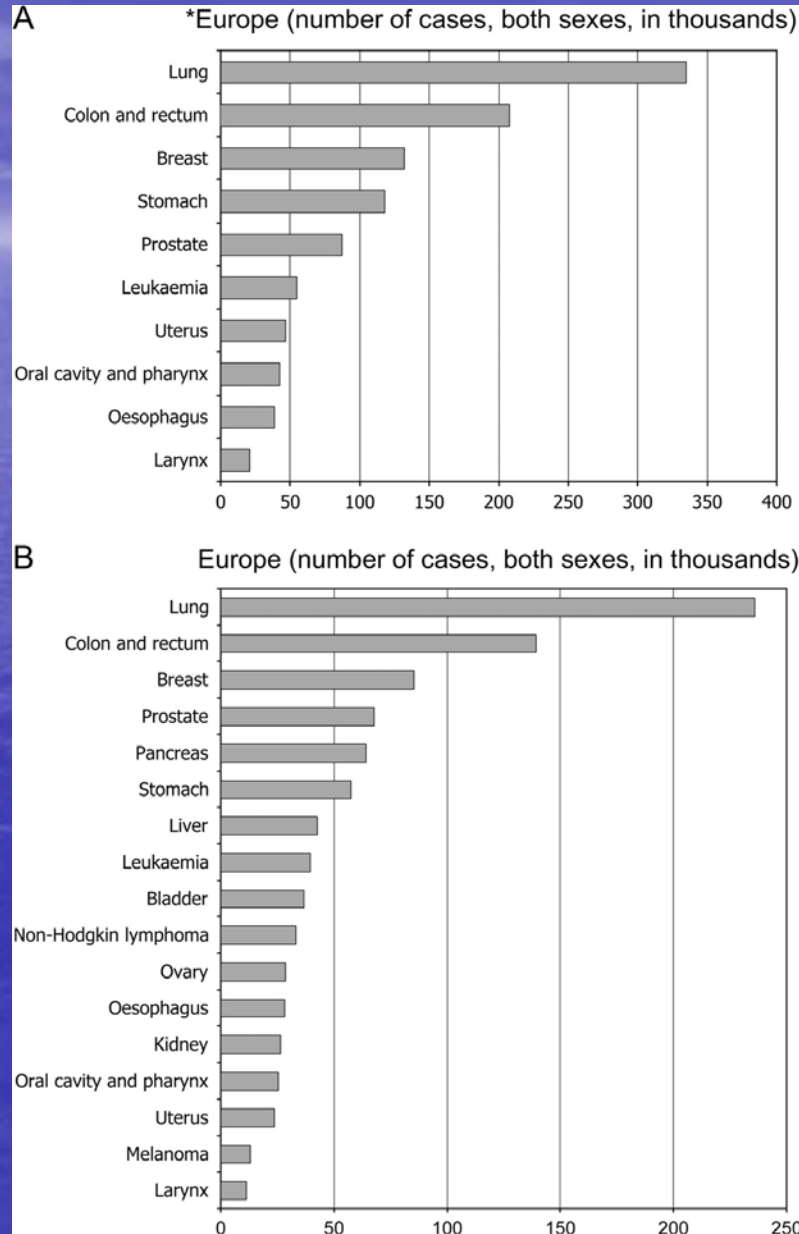
H J Roth

H Schmidt-Gayk

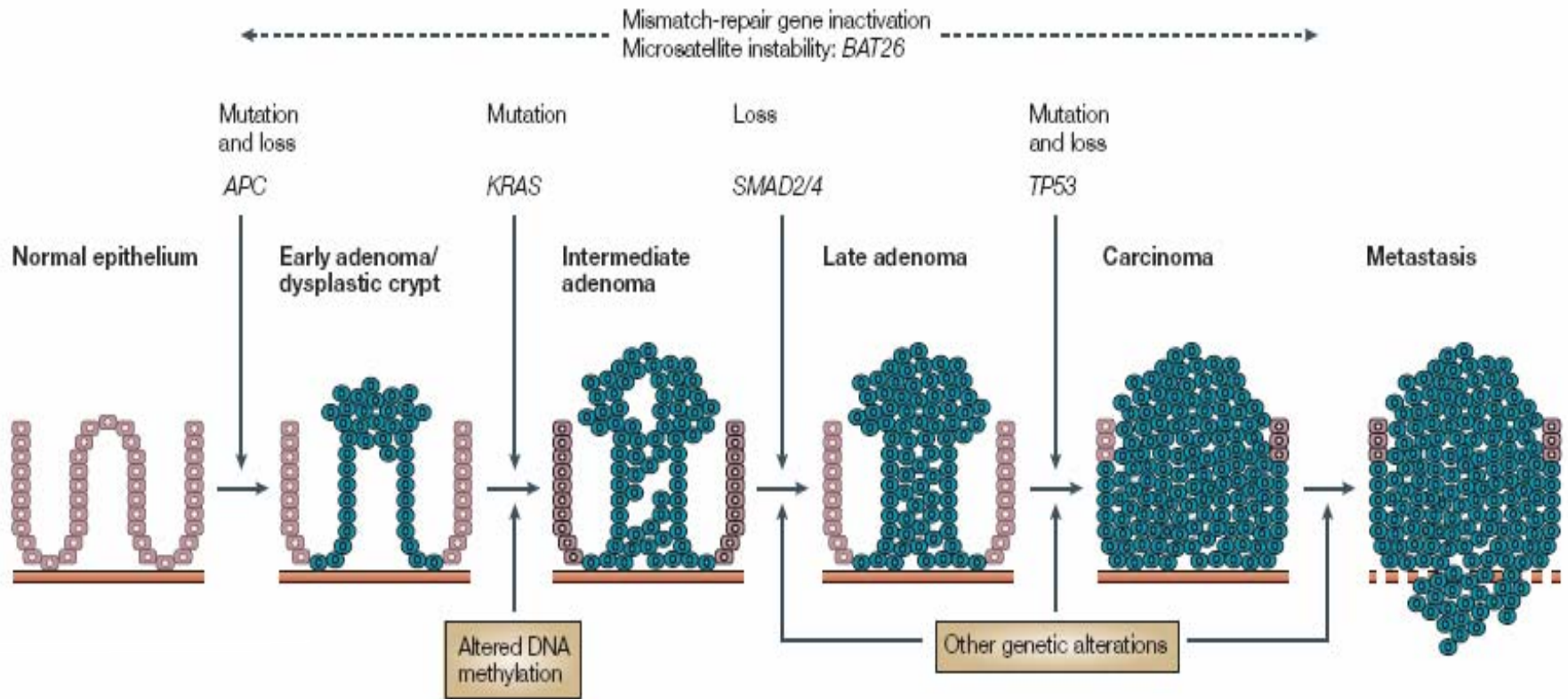
Estimated incidence of cancer in Europe and European Union, 2006



Estimated mortality from cancer in Europe and European Union, 2006

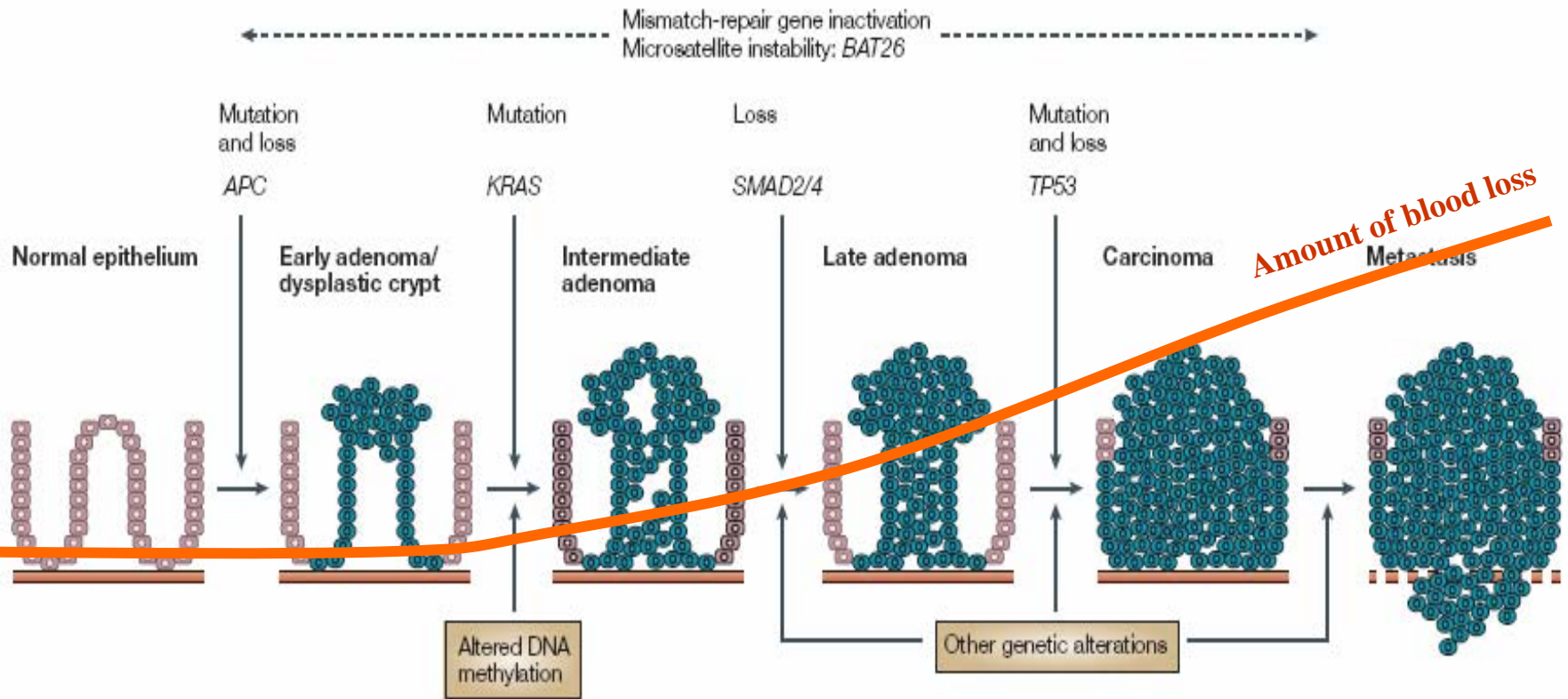


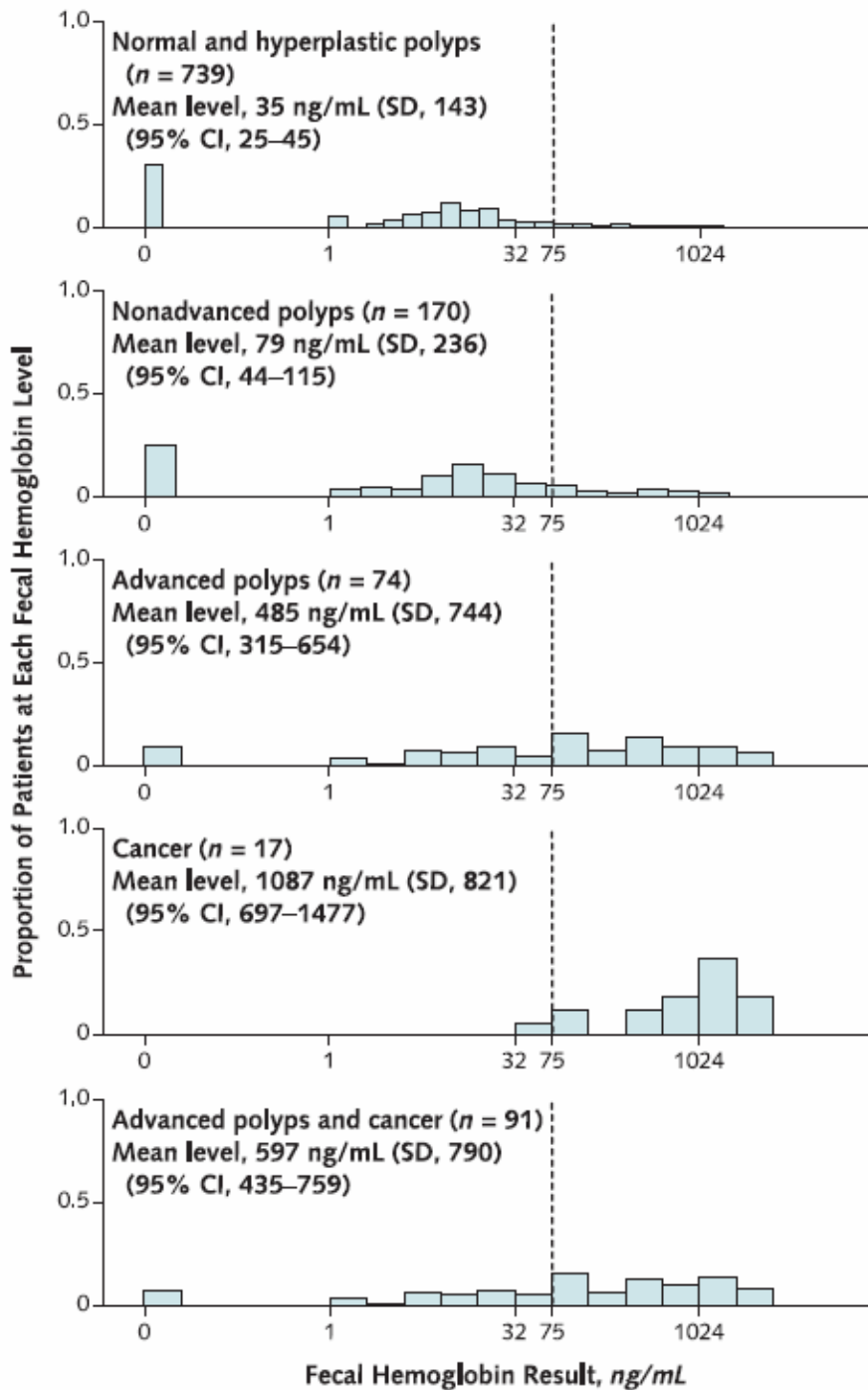
The colorectal adenoma – carcinoma sequence



5 – 10 years

The colorectal adenoma – carcinoma sequence related to occult bleeding

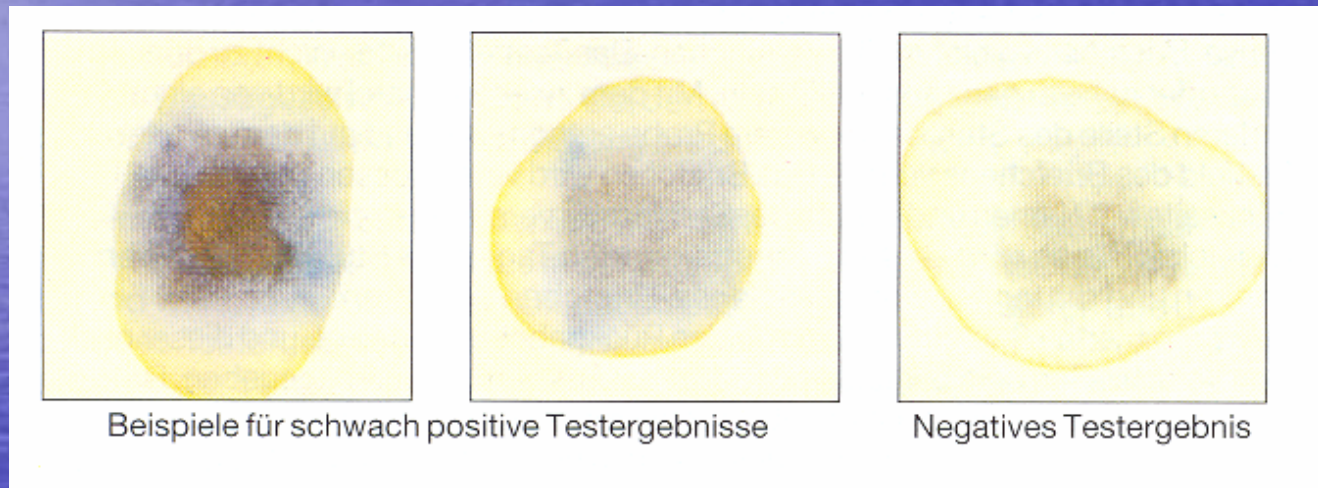




Levi Z et al.
Ann Intern Med 2007; 146:
244 - 55

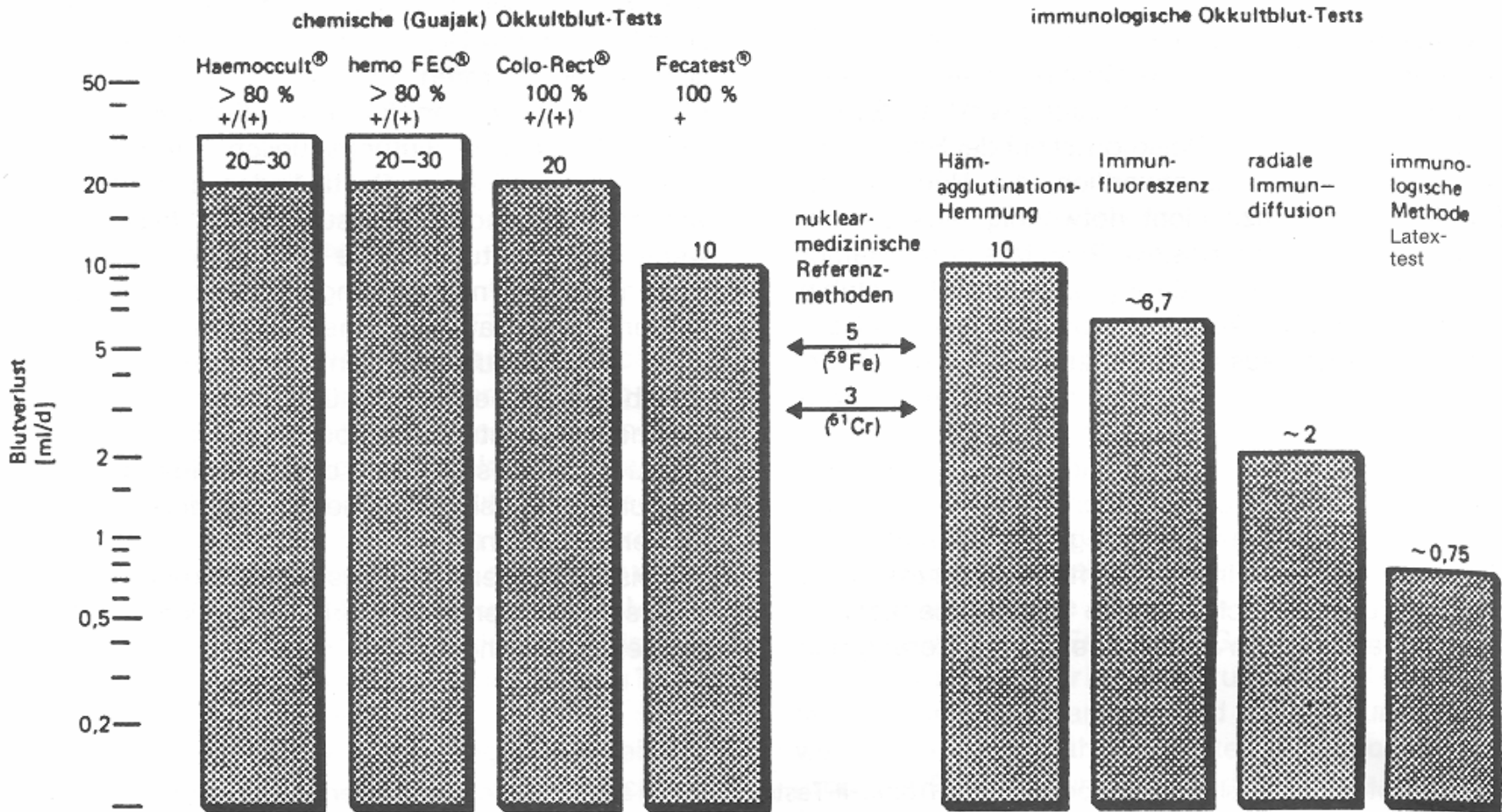
Guaiac-based versus immunochemical FOBT

- Guaiac turns blue after oxidation by oxidants or peroxidases in the presence of an oxygen donor such as hydrogen peroxidase. Peroxidase – like or pseudoperoxidase activity of heme in hemoglobin
Qualitative result.



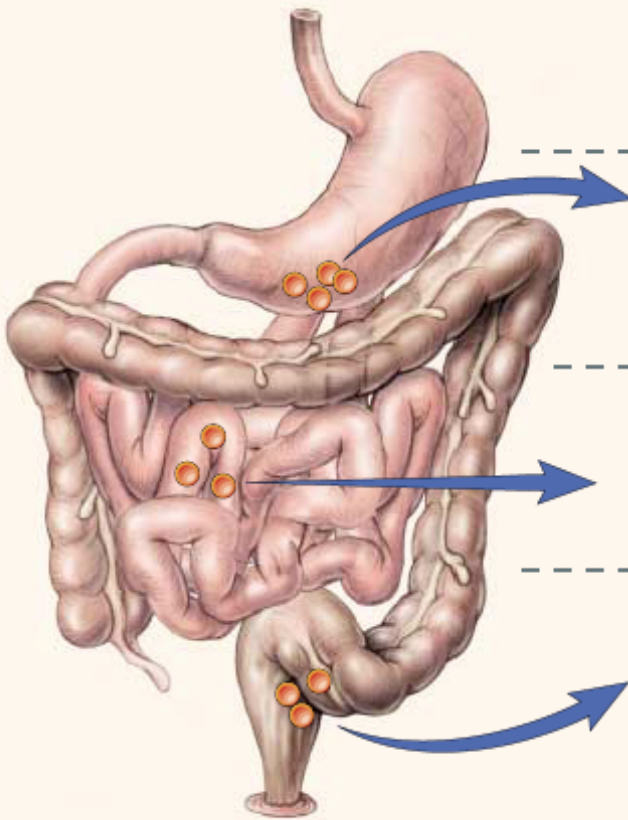
- Immunochemical tests employ poly- or monoclonal antibodies alone or in combination that detect antigenic sites on the intact globin portion of hemoglobin. Highly specific for human (hemo)globin
Quantitative result

"in vivo" detection limits of blood



Sites of Gastrointestinal Bleeding, Intraluminal Metabolism of Hemoglobin, and Accuracy of Fecal Occult-Blood Tests

Sites of Gastrointestinal Bleeding



Relative Likelihood of a Positive Fecal Occult-Blood Test

Upper gastrointestinal tract

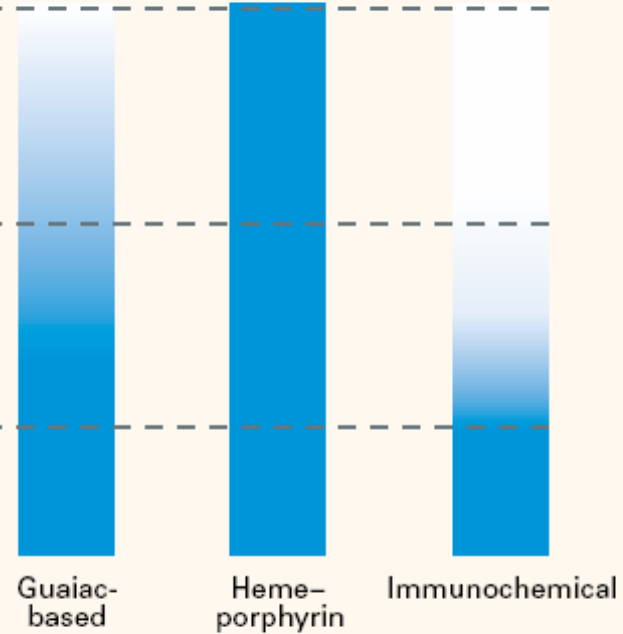
Porphyrins, partially degraded heme, degraded globin

Middle gastrointestinal tract

Porphyrins, partially degraded heme, partially degraded globin

Lower gastrointestinal tract

Intact heme and intact globin

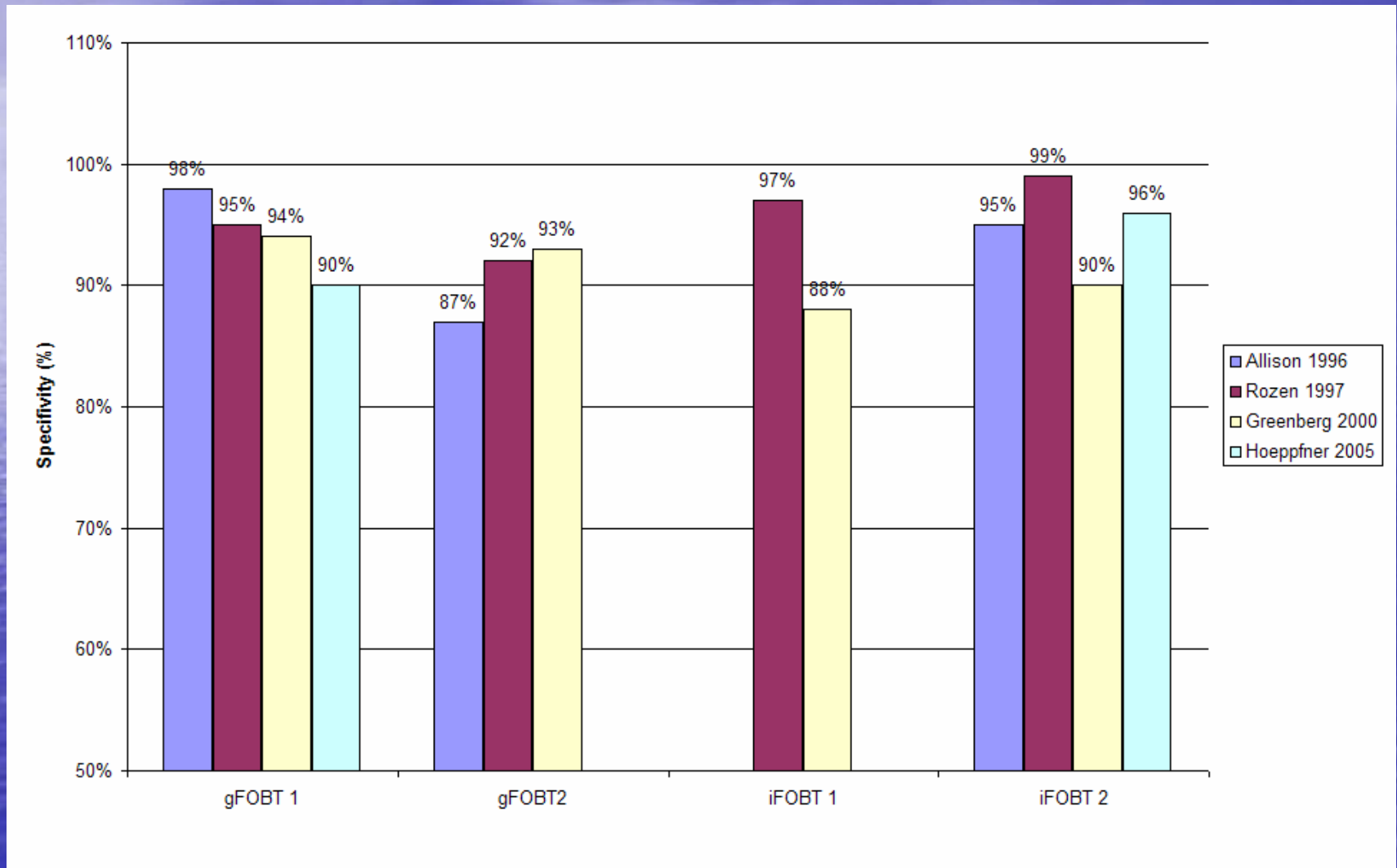


Variables influencing FOBTs

False positive	Guaiac based	Immunochemical
Nonhuman hemoglobin (myoglobin and hemoglobin from beef, horse, pig...)	++++	-
Dietary peroxidase (horseradish, radish, cauliflower..)	+++	-
Rehydration	++	-
Iron	(+)	-
False negative		
Hemoglobin degradation	++	+++
Storage (neat stool - room temperature)	++	++++
Vitamin C	++	-
Others		
Anticoagulants or aspirin	-	-
Deodorizers / fresheners / cleaners / sanitizers	-	- +

Performance characteristics of guaiac based versus immunochemical FOBTs.

Specificity

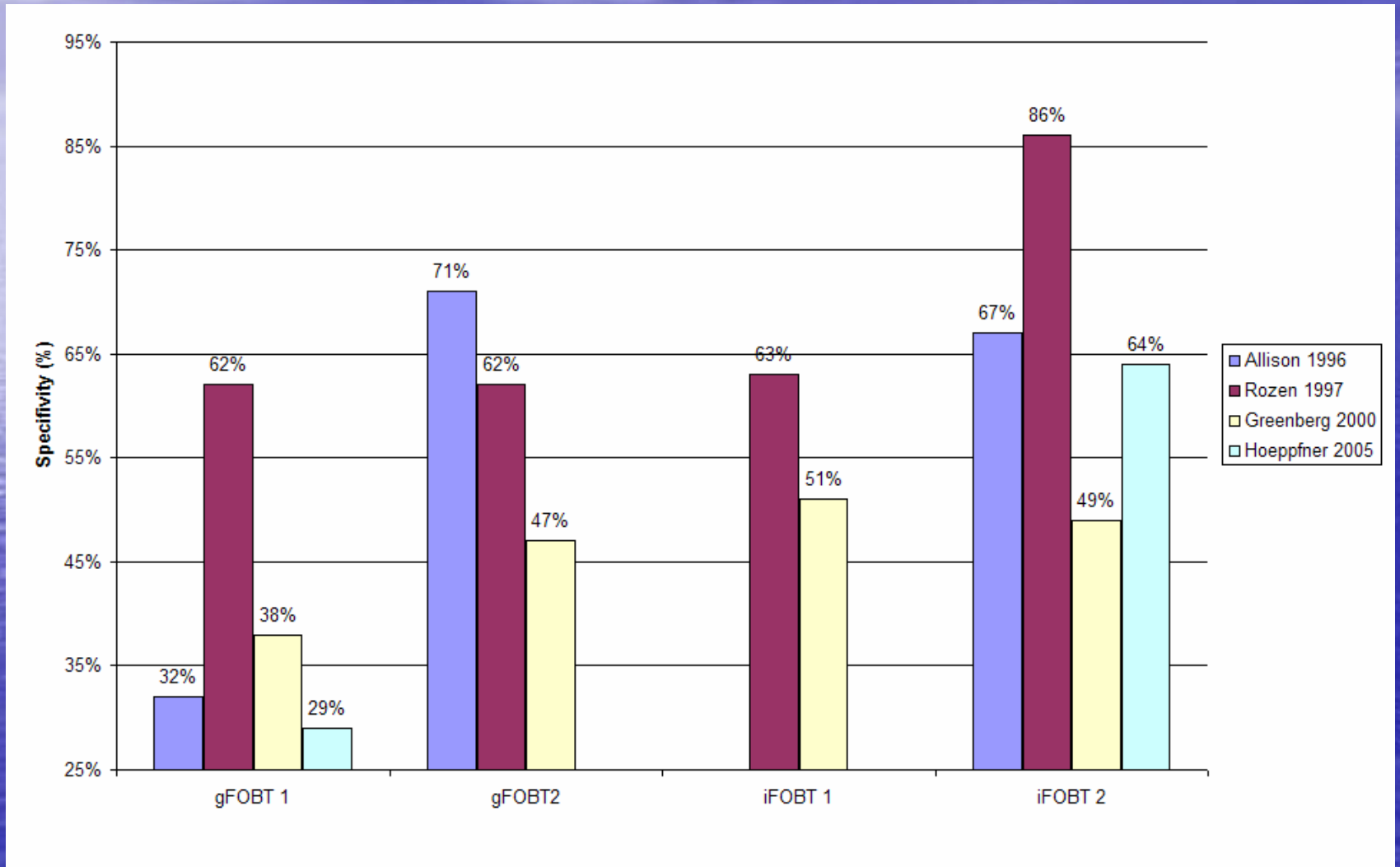


gFOBT = guaiac based

iFOBT = immunochemical

Performance characteristics of guaiac based versus immunochemical FOBTs.

Sensitivity

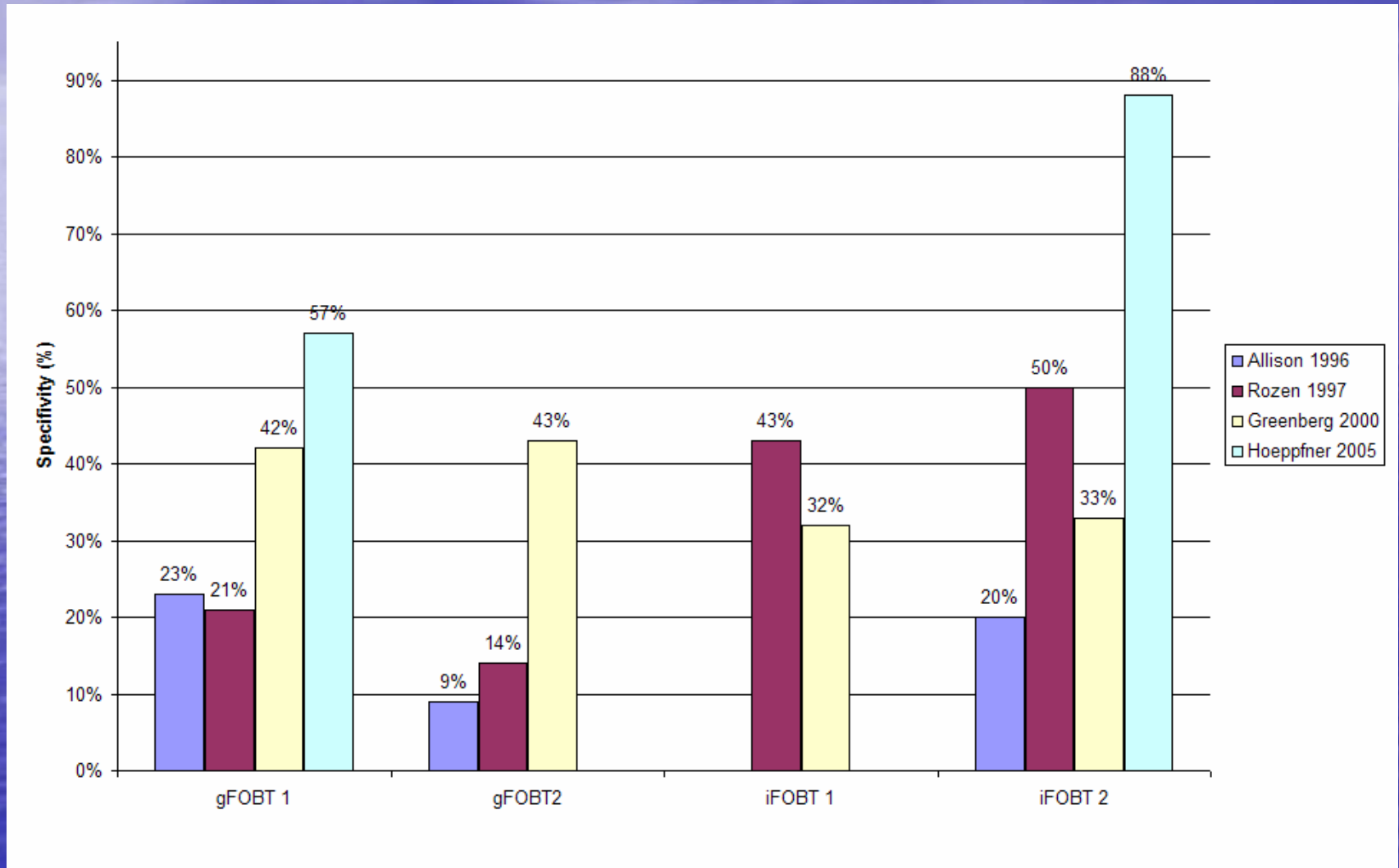


gFOBT = guaiac based

iFOBT = immunochemical

Performance characteristics of guaiac based versus immunochemical FOBTs.

Positive predictive value



gFOBT = guaiac based

iFOBT = immunochemical

Guaiac based versus immunochemical FOBT

- High variability in sensitivity and specificity data
 - related to:
 - study design
 - evaluated study population
 - employed FOBTs
 - ...
- Immunochemical FOBT is
 - is equivalent or better than guaiac based FOBT in respect to specificity
 - more sensitive in detecting CRC or advanced neoplasia

- **Smith A et al. Cancer 2006**

„..iFOBT is more sensitive for cancers and significant adenomas than a sensitive gFOBT.“

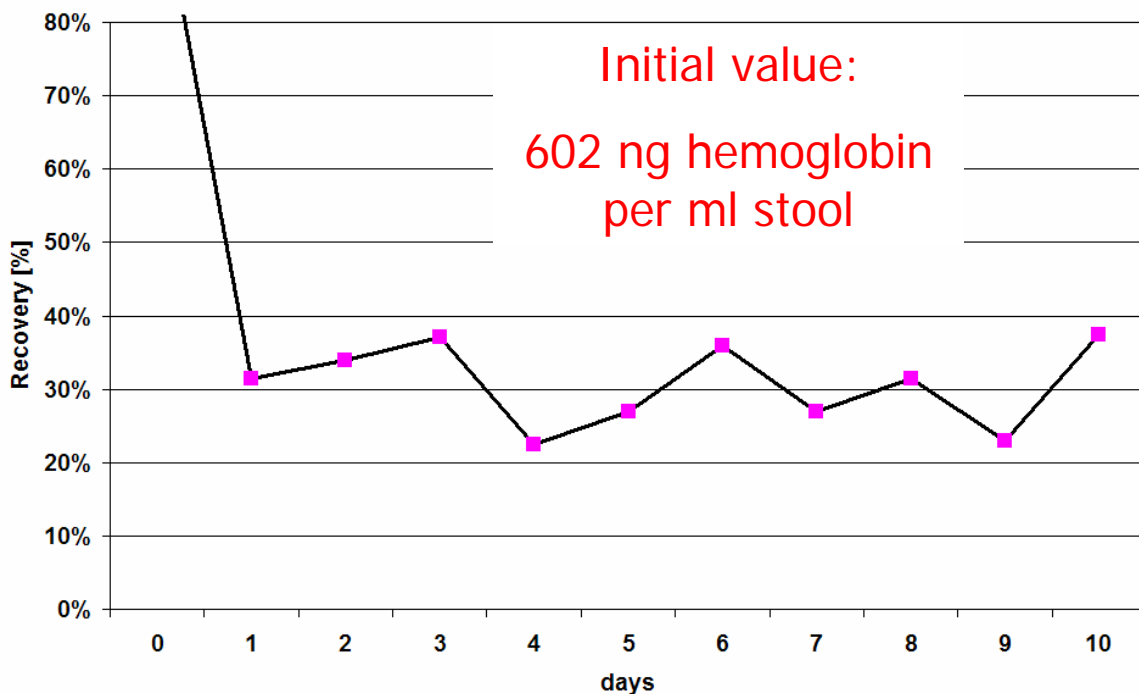
- **Guittet L et al. Gut 2007**

„With a threshold of 50 ng/ml, I-FOBT detected more than twice as many advanced neoplasias as the G-FOBT (ratio of sensitivity = 2.33) without any loss in specificity (ratio of false positive rate = 0.99). With a threshold of 75 ng/ml, associated with a similar positivity rate to G-FOBT (2.4%), the use of I-FOBT allowed a gain in sensitivity of 90% and a decrease in the false positive rate of 33% for advanced neoplasia.“

Stool sampling devices – neat stool

Neat stool – freeze immediately and keep frozen until being processed!

Significant initial loss (highly variable, up to 70% within 24h) of hemoglobin when keeping sample at room temperature.



Stool sampling devices – with storage / extraction buffer



Stool sampling device – serrated sampling tip

Reproducible and quantitative transfer of stool into the final sampling tube.

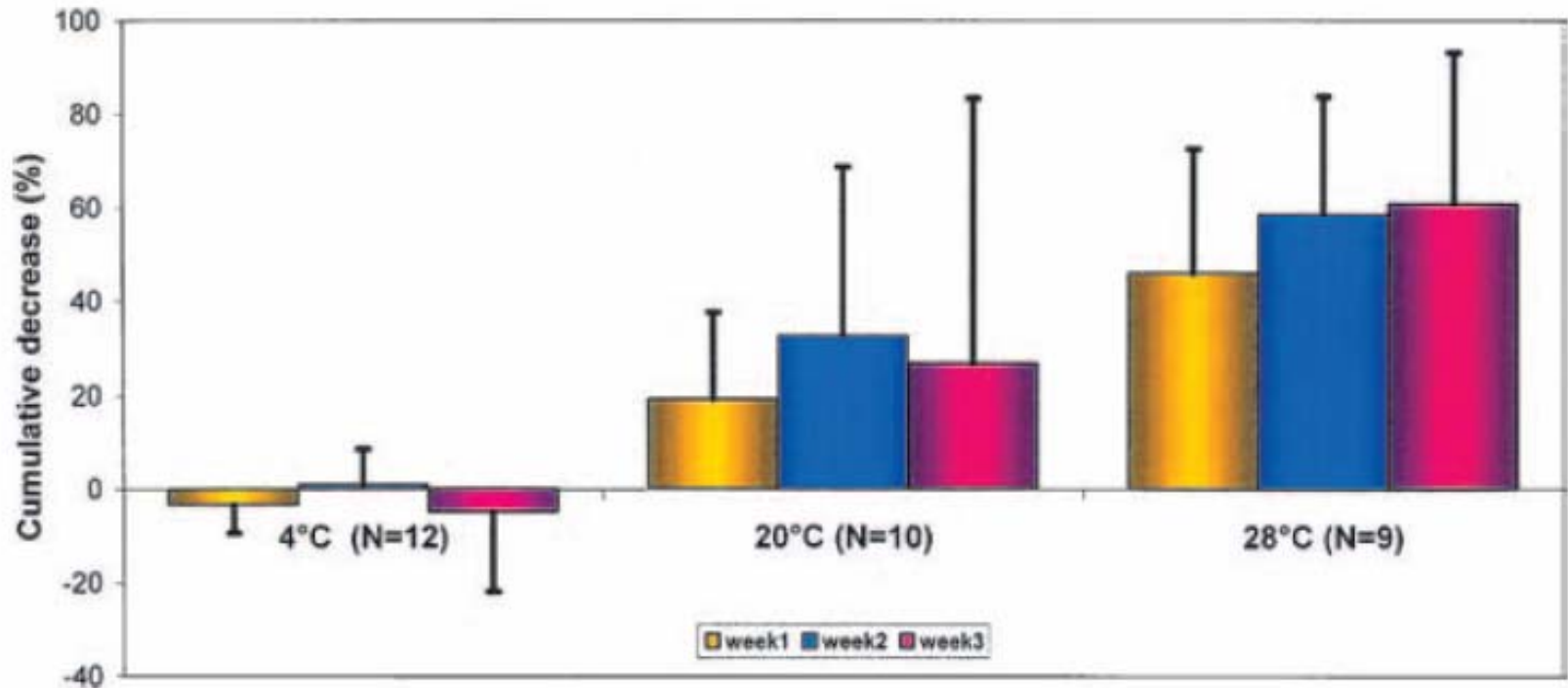
22 replicates; 11 different positions from homogenized stool:

Mean: 198 ng/mL CV : 6.15 %

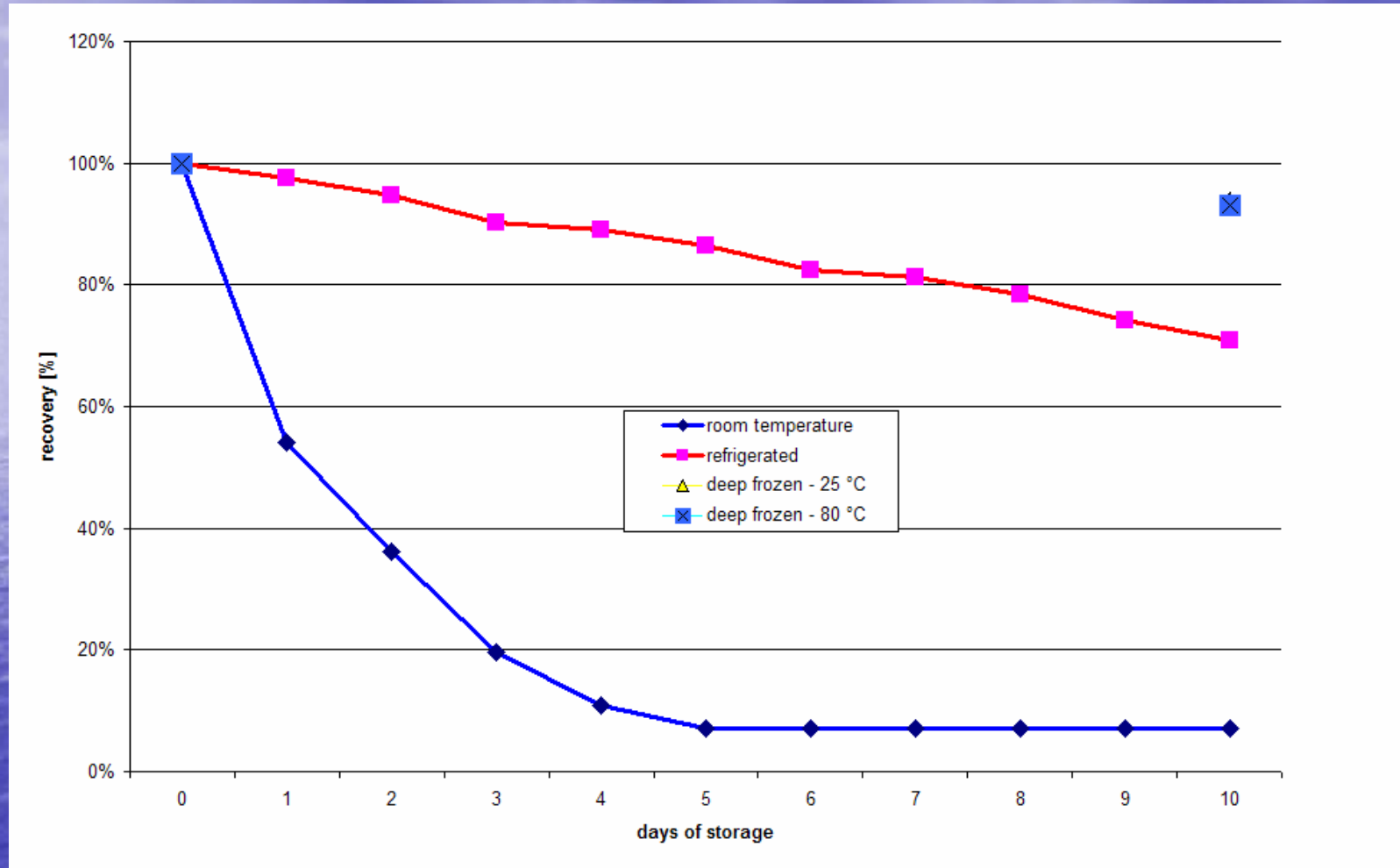
Mean: 602 ng/mL CV : 5.16 %



Stability of sample in fecal test sampling device

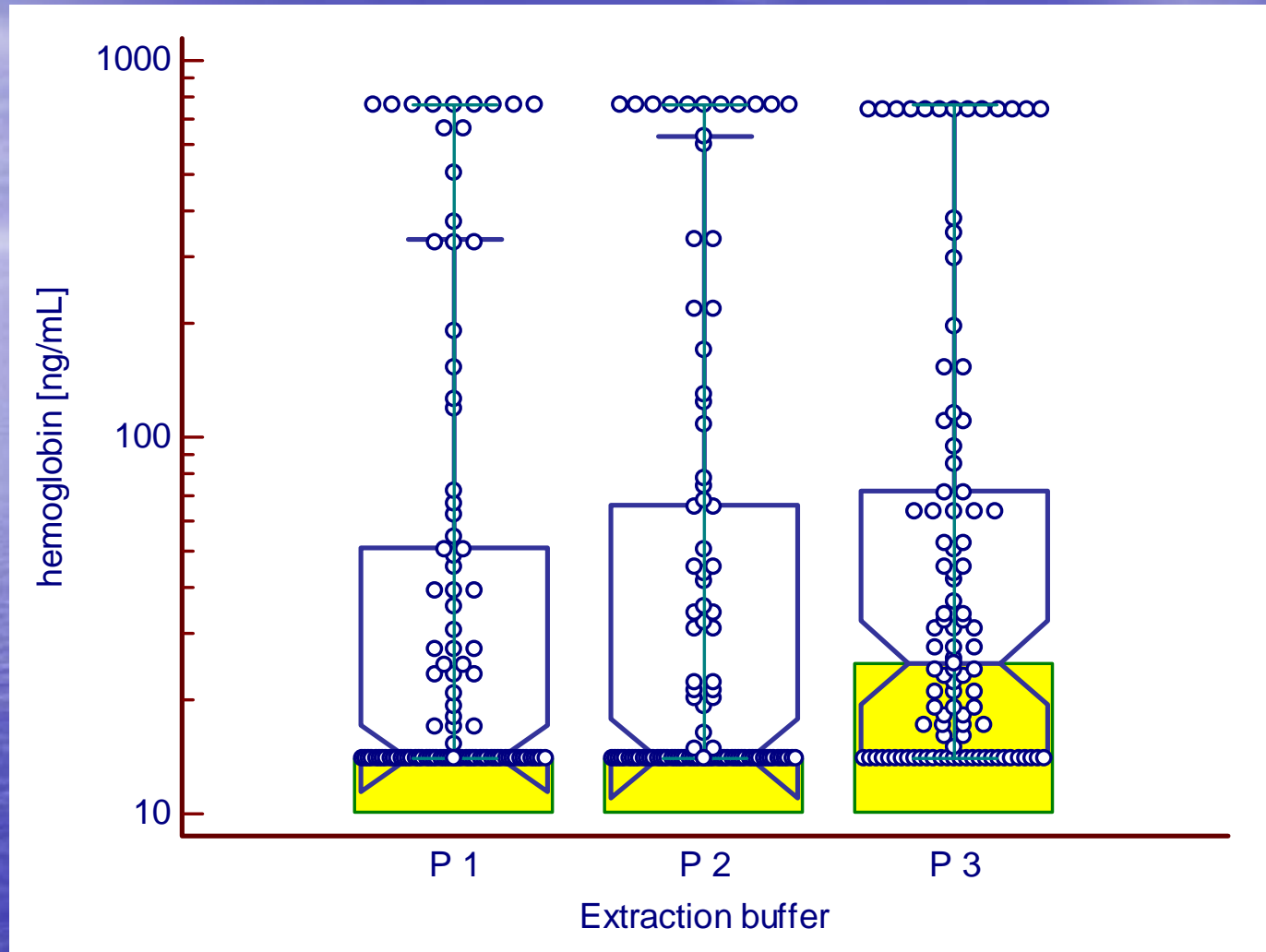


Stability of sample in fecal test sampling device



Initial mean value: 198 ng/mL

Impact of buffer composition on efficiency of hemoglobin extraction



n=100; marginal, but significant differences of measured hemoglobin concentrations (iFOBT: Sentinel Italy) related to extraction buffer composition. Buffer composition modified in respect to employed stabilizers and detergents.

Conclusion

- Higher sensitivity and specificity for iFOBT especially with more recently developed iFOBTs
- Burdensome dietary restrictions for guaiac based test and
- Simplification of sampling techniques by means of stool sampling devices (iFOBT)
 - will improve compliance in participation in CRC screening programs
- Quantitative iFOBT result will enable adjustment of specific thresholds with an ideal balance between sensitivity and specificity related to the evaluated population
- Automated processing of iFOBT will reduce variability of test results

Conclusion

- The impact of sampling device, extraction or stabilizing buffer on test performance should not be neglected
- High number (>15) of commercially available iFOBTs may have a significant (negative ?) impact on CRC screening
- Randomized controlled studies will be needed confirming obtained data especially with recently developed iFOBTs and to prove the superior behavior of immunochemical FOBT

Final study population

Study population	n=	
total	725	
Colorectal Cancer CRC		
Total	186	107 colon cancer, 79 rectum cancer
Subpopulation	100	75 colon cancer, 25 rectum cancer
Adenoma	171	
advanced neoplasia	113	
Non-advanced neoplasia	58	
Control population	257	135 GI-healthy, 29 hemorrhoids, 74 diverticulosis, 14 polyps hyperplastic, 5 non-inflammatory bowel disease
Disease controls	74	17 diverticulitis, 18 colitis, 24 inflammatory bowel disease, 6 ulcer, 3 lipoma, 6 inflammatory GI disease
Gastrointestinal cancer	37	5 esophagus, 9 liver/bile duct, 7 pancreas, 12 stomach, 2 small intestine, 2 gallbladder