

On behalf of all staff at R-Biopharm AG in Germany we would like to take this opportunity to thank you for the good cooperation throughout the past year and wish you all the best for 2007 !



Intestinal-cancer screening is still an issue in Germany

Despite a variety of initiatives by the Burda Stiftung, Stiftung Lebensblicke, Gastro Liga, and various other organizations, a great many patients still hesitate to undergo cancer-screening examinations.

Today as ever, there is still a large number of physicians who have not even discussed the possibilities to undergo intestinal-cancer screening schemes with their patients.

In Germany the number of coloscopic examinations performed in patients dropped by a double-digit percentage in 2006. At the same time there has been a resurgence in the number of only slightly sensitive guaiac tests (envelope tests) carried out. This, of course, results in fewer coloscopic examinations being performed.

Stool envelopes for the detection of occult blood in stool samples have been available ever since 1977. The technology used by these guaiac tests is accordingly no longer state-of-the-art. The drawbacks and the lack of diagnostic security entailed in this test method are broadly familiar. The ingestion of certain foods can lead to the test yielding false-positive or false-negative results. Patients with a positive result are advised to undergo colonoscopy to clarify the diagnosis.

The by far greater problem, however, is the one posed by false-negative results. These patients go to the trouble of at-

tending a screening appointment and in some cases are drawn into a false sense of security by being presented with a negative finding. And this only because the guaiac test did not result in a change in the colour due to the pseudo-peroxidase reaction being blocked or diminished by vitamin C or another antioxidant.

A far more sensitive method is given by immunological tests; the decisive factor is, however, the definition of the respective cut-off ranges. If these are set too high, in many cases the test fails to identify patients properly, meaning that they are not referred for the required coloscopic clarification. Regrettably, not all of the tests that are commercially available have been sufficiently validated from the clinical viewpoint, in other words no studies have yet been made into their sensitivity and specificity.

One aspect that is certain, however, is that each and every rectal or perianal hemorrhage constitutes an indication for colonoscopy. Occult blood in the stool is a sign that something is amiss in the patient. Even hemorrhoidal hemorrhaging does not exclude the possibility of colorectal carcinoma.

Whereas patients with colorectal tumors at an advanced stage have a poor long-term prognosis, tumors that are diagnosed very early on in their devel-



opment can be removed in most cases before they achieve metastasis. The aim is hence to identify precancerous processes at an early stage.

Early diagnosis is an important factor, since in Germany alone the annual costs of treating the disorder without the follow-on costs are currently in the region of a half-billion euros.

If patients decide not to attend coloscopic screening schemes, then the office-based physician should at least offer his/her patients the possibility of a highly sensitive screening test as a preventive-medical service. The principle of the detection of hemoglobin and hemoglobin/haptoglobin complex is, unfortunately, not familiar throughout all physicians' practices. Such immunological tests are characterized by a high sensitivity and specificity and by their ease of use.

By using mono- and polyclonal antibodies capable of detecting exclusively human hemoglobin and the hemoglobin/haptoglobin complex, these immunological tests are significantly more sensitive than conventional ones. False-negative or false-positive results due to

the effects of meals taken before the tests are excluded.

The immunological detection of hemoglobin sensitively identifies hemorrhages from colonic carcinomas – however, especially in the case of adenomas and carcinomas in the right colon, the long intestinal passage may result in the degradation of any hemoglobin present.

The complex formed by hemoglobin and haptoglobin in contrast is far more stable. This enables even hemorrhages from larger intestinal polyps and colonic carcinomas located in the upper parts of the intestinal tract to be securely diagnosed. What's more, the Hb/Hp-complex test is characterized by a significant enhancement of the sensitivity towards colorectal adenomas, depending on the size and the degree of dysplasia as high as 80 % at a specificity of approx. 95 %.

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R-Biopharm supplies kits for the detection of hemoglobin and hemoglobin/haptoglobin complex as a double rapid test and soon also as an enzyme immunoassay .



Clostridium perfringens Enterotoxin (CPE)

While *Clostridium difficile* has recently come to enjoy the medical limelight thanks to numerous reports of particularly virulent strains and an associated rise in mortality, *Clostridium perfringens* still occupies a back seat in the clinicodiagnostical area in Germany.

This is surely unjustified, as a multitude of publications and current research results go to prove.

In a recently published Finnish study, for example, it was shown that *C. perfringens* strains carrying the gene for the formation of enterotoxins were found in 18 % of all stool specimens obtained from healthy grocers. This is evidence enough that the human being constitutes a major reservoir

for this anaerobic spore-forming bacterium.

An English study carried out in 2002 in 200 patients suffering from antibiotic-associated diarrhea (AAD) revealed that 8 % of them hosted *C. perfringens* enterotoxin (CPE), 16 % *C. difficile* toxin A/B, and 2 % toxins of both pathogens.

A very recently published prospective study conducted by the same English



working group among 4,659 hospitalized patients revealed, in those patients in this group suffering from AAD, an incidence of 12.7 % for *C. difficile* toxins A and B and one of 3.3 % for *C. perfringens* enterotoxin.

On account of the ubiquitous spread of *C. perfringens* in the intestine of many domestic and wild animals, but also in the human intestine, special attention should be devoted to the nosocomial dissemination of this pathogen. After all, AAD triggered by CPE results in longer periods of hospitalization of the patients affected to the same extent as the cases of AAD due to *C. difficile*. A clear-cut diagnosis would enable *C. perfringens* to be rapidly and effectively treated with metronidazole and patients to be discharged from hospital care sooner.

The research carried out over the past 20 years since the connection between AAD and sporadic diarrhea (SD) and the detection of CPE in stool specimens of the affected patients was first described has yielded a wealth of new findings about the pathogen. For example, CPE has never been identified as a factor in connection with the hazardous pseudomembranous colitis (PMC), which always constitutes a raised risk for morbidity and mortality in connection with an infection with *C. difficile*.

The potential for food intoxication due to *C. perfringens* has been known for a long time and on account of the frequency of their occurrence cases of food poisoning enjoy considerable media attention, despite the relatively mild courses of the clinical symptoms. In this connection, the clinico-diagnostic identification of CPE in the stool specimens of the patients affected is attempted only in very few cases.

Thanks to molecular-biological analysis, we today know that *Clostridium perfringens* forms both chromosomally as well as plasmide-coded enterotoxin. In this connection it was shown that all strains capable of causing food poisoning excrete without exception chromosomally formed CPE during the sporulation phase, whereas strain isolates gained from patients with AAD or SD form only plasmide-coded enterotoxin. Strains with this episomally formed enterotoxin are, however, pathogenetically far more effective than the disorders caused by intoxicated foods and result in some cases very pronounced courses of disease. These more severe and more protracted courses are presumably connected with the general state of health

of the carriers of such *C. perfringens* strains. Either there are already underlying illnesses present, or the patients affected are relatively old and/or have a weakened immune defence.

RIDASCREEN® Clostridium perfringens Enterotoxin ELISA



In particular residents of old people's homes and hospitalized patients receiving antibiotic therapy as a special risk factor have a raised susceptibility for colonization with pathogenic strains of *C. perfringens*. In this regard *C. perfringens* deserves to be awarded the same attention as *C. difficile*.

Even if we still do not know everything about the mechanisms and conditions that result in the expression and release of toxins, and also know relatively little about the constancy of the plasmide-coded CPE formation process, under no circumstances we should neglect the second most frequent pathogen for AAD.

Since the enterotoxin is capable of stably persisting in stool specimens for 2 days at 2 - 8 °C and for a longer time at -20 °C, CPE diagnosis can also be readily used as a "second line" diagnostic method following a *C. difficile*-toxin A/B-negative finding, especially under the consideration that is more than just sensible from the viewpoint of differential diagnostics.

The RIDASCREEN® Clostridium perfringens Enterotoxin ELISA, next to the well-established *Clostridium difficile* Toxin A/B ELISA test, constitutes an essential and above all else a system for the rapid identification and clarification of hitherto underdiagnosed other causes of AAD or SD. A dependable and clear-cut diagnosis is, of course, essential for making the correct therapeutical decision and also serves to ensure that patients recover their health more swiftly.

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Death of a newborn child in Thuringia, Germany after primary maternal HSV-1 infection in the late phase of pregnancy

Although the relevance of infection with Herpes neonatalis with in many cases a lethal outcome when left untreated is known, even in highly developed countries the danger posed by infection with HSV during pregnancy is frequently ignored. In this connection, the investment of just a few euros in appropriate diagnostic methods may clarify the hazard potential for both the mother-to-be and her unborn child, and a corresponding management according to the international recommendations (International Herpes Management Forum; www.ihmf.org) could be initiated.

In the present case, shortly before term a pregnant woman was diagnosed as having fever followed by a rubella-similar exanthema. These symptoms notwithstanding, no virological diagnostic procedures were initiated. The birth took a normal course, and the newborn child exhibited no clinical symptoms. On the sixth postnatal day the neonate was admitted to a local hospital with an exanthema and indications of jaundice. On the basis of a 1,000-fold increase in the transaminase values, on day nine the child was referred to a hospital with an neonatal intensive-care unit. Here the condition of the child deteriorated dramatically over the next few days, resulting in its death on day 13.

It was the pathologist who first considered the possibility of an HSV infection. PCR was used to identify infection with HSV-1 as the underlying cause. Type-specific serological diagnostic methods employing RIDASCREEN® HSV-1 IgG EIA also revealed that neither the mother nor her newborn child displayed any IgG antibodies against HSV-1, while HSV-2 IgG was detected. The mother was hence already infected with HSV-2, which goes to prove yet again that an existing infection with one HSV type does not afford protection against the fatal consequences of a primary infection with the other type. The seroconversion of the mother regarding HSV-1 was detected on day 41 after delivery, proving that the mother must have become infected with HSV-1 in the late phase of pregnancy.

If the possibility of an HSV infection of this newborn child had been considered in good time, then this could have been properly diagnosed by means of PCR, and therapy with aciclovir would have prevented the lethal outcome. This notwithstanding, measures would have had to be taken to prevent the infection of the unborn child. If an adequate diagnosis involving antigen and antibody detection had been carried out at the time the mother-to-be presented with

the febrile exanthema, the primary infection with HSV-1 would have been discovered. A positive HSV-1 result with negative serological findings is indicative of a primary infection.

In this connection, however, the detection of type-specific antibodies – which is possible using the RIDASCREEN® HSV EIAs – is of particular importance. Many commercially available tests are based on the full antigen of HSV-1 and HSV-2. Due to the presence of cross-reactive antigenic determinants, however, these do not actually detect type-specific antibodies. In the case described here, the use of such a test in connection with the existing HSV-2 infection of the mother-to-be may have produced a false-positive result and thus resulted in a misinterpretation of the present risk. Due to the lack of maternal antibodies, a primary infection always entails an extremely high risk for the unborn child. Corresponding precautionary measures for the delivery of the child should always be taken in the management of pregnancy.

If, however, seroconversion is already complete in the mother-to-be, the risk for the unborn child becomes considerably reduced. A type-specific detection of antibodies can hence contribute to a positive prognosis and to a relaxation of what is initially a highly critical situation.

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Literature

Meerbach A, Sauerbrei A, Meerbach W, Bittrich HJ, Wutzler P: Fatal outcome of herpes simplex virus type 1-induced necrotic hepatitis in a neonate. *Med Microbiol Immunol* (2006)



German Ministry for Education and Research (BMBF) sponsors tumour research at R-Biopharm

Since April and August of this year, two major research projects in the field of tumour diagnosis – “BioChance Plus” and “TumorVision” – are being sponsored by the BMBF to the sum of almost 2.16 million euros over a period of three years. With these two R&D projects in the area of clinical diagnosis, R-Biopharm – which has already been among the top 50 of the fastest growing companies in Germany several times – has succeeded in positioning itself for the very first time in the cutting-edge group of research-intensive diagnostics suppliers, investing more than 8% of its total revenues in the development of future-oriented products.

The “BioChance Plus” project is not only focussing on the development of diagnostic test systems for the detection of transketolase-like 1 protein

(TKTL1) from various physical materials such as blood, stool, and urine, but also on that of a TKTL1-specific substrate for positron emission tomography (PET) as the central aspect of this user-oriented development. On top of this, Dr. Johannes F. Coy, the discoverer of TKTL1 and DNaseX, is heading a subproject to further develop a TKTL1 inhibitor into an anti-cancer drug.

With its RIDA® PentoCheck® IHC, since the beginning of 2006 R-Biopharm has been supplying the first CE-certified test for the immunohistochemical identification of TKTL1 from biopsy and resection specimens of tumours of all kinds. The highly specific monoclonal murine antibody against human TKTL1 has already been highly successfully tested in more than 20 different entities of solid tumours as well as in melanomas, in tumours of the brain, and in leukemia. In each of the tumour entities tested there was a subgroup with TKTL1-positive tumours. For a wide number of various tumour entities – including a series of high-incidence forms of cancer, such as e.g. intestinal cancer, cancer of the bladder, breast cancer, ovarian cancer, and even lung cancer – it was possible to detect a statistically significant relationship between the expression of TKTL1 (staining by immunohistochemistry) and the aggressiveness of the tumour and also the long-term prognosis of the patients affected.

Patients with highly TKTL1-positive tumours have a considerably shorter

postoperative survival prognosis than do patients with TKTL1-negative or weakly positive tumours.

For the second BMBF-sponsored project, “TumorVision”, which as a joint project involves industrial and academic cooperation partners such as the supplier of endoscopy products Karl Storz, Tuttlingen; the laser-research laboratory of the State Medical University of Munich; the Institute for Analytical Chemistry of the University of Regensburg (ACUR); and the Regensburg University Clinic as well as a number of further subcontractors, R-Biopharm has agreed to accept the task for the overall project coordination. The aim of this interdisciplinary project is to enable the fluorescence-mediated detection of marker enzymes for the in vivo visualization of tumours and their precursors using endoscopic methods. Assuming that these ambitious targets of the project will be achieved, the “in vivo” identification of DNaseX and TKTL1 may revolutionize the diagnosis of cancer, above all in the gastrointestinal tract, but also in the oropharyngeal area and in the lung.

One aspect is already clear to everyone involved: in addition to the immense intellectual and scientific challenges that the cooperation in these BMBF projects involves, research into TKTL-1 may trigger the paradigm switch in the diagnosis and therapy of cancer originally predicted in the 1920s by the German biochemist and Nobel Prize Laureate Otto Warburg.

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Trade Fairs and Conferences

19.01. - 20.01.	ZAEN congress Freudenstadt, Germany
11.02. - 14.02.	Arab Lab 2007 Dubai, UAE
09.03. - 10.03.	ZAEN congress Düsseldorf, Germany
21.03. - 22.03.	PentoCheck® International Training Darmstadt, Germany
31.03. - 03.04.	17. ECCMID, International Congress Center Munich (ICM) Munich, Germany
02.04. - 05.04.	BelarusMedica 2007 Minsk, Belarus
21.05. - 25.05.	R-Biopharm Distributor Training Kiev/Lviv, Ukraine

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