Comparison of gene expression data from homogenized and lysed patient tissue derived from either unstained or hematoxylin and eosin (H&E) stained slides shows a high correlation (r=0.98). This provides an advantage when studying heterogeneous tumours that are microdissected from H&E stained slides. In fact, using this methodology, an estrogen receptor-positive tumour was analysed and one of the tumour foci had a more advanced tumour expressing the mesenchymal marker, FN1 (fibronectin). This was only possible by running a 40-plex assay on minimal input material (microdissected from 20 μm section) representing markers for molecular classification, epithelial to mesenchymal transition, and proliferation markers [7]. A recent audit on breast cancer diagnosis, indicates clearly that heterogeneous cases characterized using the bead-based multiplex assays on resection tumour samples are not represented in matched biopsies used for patient diagnosis. In fact, only 3.5% of 97 intra-tumour heterogeneous cases were detected in a cohort of 570 patients at diagnosis. The advantage of the digitalized result of the Innoplex assays is to avoid increasing the workflow of pathologists when resected samples are re-analysed to characterize multiple sites within a tumour.

**Summary**

In conclusion, the innovative multiplex assays indicate a shift from reactive medicine (treating patients based on average risk) towards predictive, precise and personalized treatment that takes into account heterogeneity of primary tumour, progression of tumour during therapy and the metastatic surveillance of the individual patient. The versatility of the method allows the development of various assays to support different applications (Figs 1 & 2). Our first innovative methods were developed for the molecular classification of luminal and basal breast cancer and to predict sensitivity to specific therapy in triple-negative breast cancer subtype [8]. As discussed above, the multiplex assays have a wide range of possible applications in the diagnosis of tumours and surveillance of tumours during therapy. The main advantages of these methods include:

- Implementation of high-throughput analysis which has a positive impact on remote testing and implementation of such assays in patient surveillance and clinical trials;
- The digitalized result excludes subjectivity and equivocal interpretation, which are common events in image-based measurements, and also eliminates the need for highly specialized facilities and human resources;
- The accuracy and precision of multiple targets in one assay, minimizing the use of precious patient samples; and
- The digitalized measurement of gene expression in heterogeneous tumours and low input/low quality patient material.

The method is streamlined with the current pathology laboratory practices resulting in a workflow that is cost-effective and with minimal turnaround time.

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**References**


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**Lactose intolerance**

Primary lactose intolerance is a genetically caused deficiency of the enzyme responsible for splitting lactose into its constituent sugars glucose and galactose. In affected patients, undigested lactose is fermented in the ileum and large intestine, producing by-products such as short-chain fatty acids, methane and hydrogen, which cause the typical symptoms of abdominal pain, nausea, meteorism and diarrhoea. Secondary manifestations include deficiencies, for example of vitamins, and as a result unspecific symptoms such as fatigue, chronic tiredness and depression.

Lactose intolerance represents the natural state in mammals: Lactase activity decreases after weaning and in adulthood is often only a fraction of the activity in infancy. Some humans, however, retain the ability to metabolize lactose into adulthood due to specific genetic variants. The frequency of lactase persistence is around 35% worldwide, although it varies greatly between different population groups. It is prevalent in regions with a long tradition of pastoralism and dairy farming, for example in Europe and in populations of European descent. In large parts of eastern Asia, on the other hand, almost 100% of the population is lactose intolerant.

In addition to the primary genetically caused form of lactose intolerance there is also the secondary acquired form. This develops as a result of damage to the intestine, for example from other gastrointestinal diseases such as Crohn’s disease, coeliac disease, infections or injury from abdominal surgery. The two forms need to be distinguished diagnostically because of the need for different treatment regimes. Whereas individuals with primary lactose intolerance must adhere to a lactose-free or low-lactose diet for life or alternatively take lactase supplements, those with secondary lactase intolerance need only restrict their dairy intake until the intestinal epithelium has regenerated through treatment of the underlying cause.

**Diagnosis of lactose intolerance**

Classic diagnostic tests for lactose intolerance are the hydrogen breath test and blood glucose tests, with which the patient’s ability to metabolize lactose is examined. However, these tests have low specificity and sensitivity and are influenced by individual factors such as the composition of intestinal flora, colonic pH, gastrointestinal motility and sensitivity to lactose fermentation products. Moreover, they cannot distinguish between the primary and secondary forms.
of lactose intolerance. Molecular genetic testing complements these methods, enabling verification or exclusion of primary lactase intolerance with high probability, as well as differentiation of the primary and secondary forms. Genetic testing is, moreover, a non-invasive and more comfortable examination, which does not carry the risk of provoking symptoms of lactose intolerance in non-lactase-deficient individuals.

**LCT polymorphisms**

The main mutations associated with lactase persistence are LCT-T391K, and LCT-22081G, which are located in the regulatory region of the lactase gene. According to current knowledge, homozygous carriers of the wild-type variants LCT-391R, and LCT-22081R develop lactose intolerance, while heterozygous carriers of the variants LCT-391K, and LCT-22081G show corresponding symptoms in stress situations or when eating lactose-containing foods. Homozygous carriers of the mutant variants LCT-391S, and LCT-22081S are lactase tolerant as adults. These two polymorphisms are strongly coupled.

**Hereditary fructose intolerance**

HFI is caused by mutations in the gene for aldolase B, an enzyme essential for fructose metabolism. The mutations result in a reduction or loss in activity or stability of aldolase B, which is responsible for catalysing the breakdown of fructose-1-phosphate (F-1-P) to dihydroxy acetone phosphate and glyceraldehyde. The toxic intermediate F-1-P then accumulates in the body, causing symptoms such as nausea, vomiting and digestive disorders and in the longer term liver damage. HFI is a rare disease, occurring, for example, with a prevalence of 1 in 20,000 in Europe. It manifests already in childhood, but may remain undiagnosed due to patients’ natural dislike of sweets, fruits and vegetables.

In addition to HFI, intolerance to fructose can also be caused by deficits in the transport of fructose into the enterocytes. This form is known as intestinal fructose intolerance or fructose malabsorption. It is much more common than HFI, occurring with a prevalence of about 30%. It is important to distinguish HFI from fructose malabsorption, because of the resulting difference in dietary requirements. Patients with HFI must completely eliminate fructose and its precursors (e.g. sucrose, sorbitol) from their diet to prevent damage to their organs. Patients with fructose malabsorption, however, should follow a fructose-restricted diet.

**Diagnosis of HFI**

Intolerance to fructose is usually diagnosed by means of the hydrogen breath test, in which a defined amount of fructose is ingested and then the amount of hydrogen in the exhaled air is measured. In patients with HFI, however, the intake of fructose carries the risk of a severe hypoglycaemic reaction. Therefore, a molecular genetic test for HFI should always be performed before a fructose load test. Early diagnosis of HFI is particularly important to avoid permanent damage to the liver, kidney and small intestine.

**ALDOB mutations**

In Europe the most frequent mutations associated with HFI are the amino acid substitutions A149P, A147D, N33K (in Human Gene Database nomenclature) and the deletion del464 in the aldolase B gene. For HFI to manifest, both alleles of an individual’s DNA must be affected by a mutation. In homozygous genotypes, the two alleles contain the same mutation (paternal and maternal inheritance). If the two alleles exhibit different mutations, this is referred to as a compound heterozygous HFI genotype.

**Parallel genetic analysis**

Molecular genetic determination of the polymorphisms associated with lactase intolerance and HFI enable diagnosis of these genetic conditions with high certainty. The EUROArray Lactase/Fructose Intolerance Direct enables simultaneous detection of the lactase-intolerance-associated polymorphisms R391K and -22081G, and the HFI-associated mutations A149P, A147D, N33K and del464. Thus, the two genetically caused metabolic disorders can be assessed with a single test.

The test can be performed on whole blood samples, eliminating the need for costly and time-consuming DNA isolation. In the test procedure (Fig. 1), the sections of DNA containing the alleles are first amplified by multiplex PCR using highly specific primers. During this process the PCR products are labelled with a fluorescent dye. The PCR mixture is then incubated with a microarray slide containing immobilized DNA probes. The PCR products hybridize with their complementary probes and are subsequently detected via the emission of fluorescence signals. The data is evaluated fully automatically using EUROArrayScan software (Fig. 2), and in the case of positive results, homozygous and heterozygous states are differentiated. Numerous integrated controls ensure high reliability of results, for example, by verifying that there are no other rare mutations in direct proximity to the tested positions which could interfere with the analysis.

**EUROArrays for infection diagnostics**

Multiplex direct detection of HPV, STI and dermatophytes

**Studies on blood donors**

The performance of the EUROArray was investigated using 116 pre-characterized samples from blood donors in Germany and from quality assessment schemes. The EUROArray revealed a sensitivity of 100% and a specificity of 100% with respect to the reference molecular genetic method.

**Conclusions**

Diagnosis of gastrointestinal disorders often involves a long and challenging process of diagnostic tests and restrictive diets. Since lactose and fructose are widely consumed by many diets, it is important to consider intolerance to these sugars during the diagnostic workup. Simple genetic analysis enables primary lactose intolerance and HFI to be confirmed or excluded as the cause of gut problems. The parallel analysis offered by the EUROArray enables especially fast and effective diagnostics. Patients diagnosed with these genetic conditions can promptly adapt their diets to ease their symptoms. If the analysis is negative, the physician can focus on searching for other causes of the digestive complaints. The molecular genetic analysis thus provides valuable support for the gastroenterology clinic.

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The main mutations associated with lactase persistence are LCT (13910G) and LCT (22018G), which are located in the regulatory region of the lactase gene. According to current knowledge, heterozygous carriers of the wild-type variants LCT (13910G) and LCT (22018G) develop lactose intolerance, while heterozygous carriers of the variants LCT (13910G) and LCT (22018G) only show corresponding symptoms in stress situations or with intestinal infections. Heterozygous carriers of the mutant variants LCT (13910A) and LCT (22018G) are lactase tolerant as adults. These two polymorphisms are strongly coupled.

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**EUROArrays for infection diagnostics**

Multiplex direct detection of HPV, STI and dermatophytes

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- Comprehensive detection of different infectious agents in a single reaction
- Simple test performance with ready-to-use reagents
- High result security due to various integrated controls
- Fully automated standardised evaluation and result documentation
- LIMS connection available

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**Test system**

- **EUROArray HPV**
  - Detection and typing of all 33 relevant high and low-risk oncogenic HPV for cervical cancer prevention
- **EUROArray STI**
  - Detection of 11 relevant sexually transmitted pathogens (bacteria, viruses, fungi)
- **EUROArray Dermatology**
  - Detection of 8 dermatophytes plus specific identification for 21 dermatophytes and 8 yeasts

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**Figure 1. EUROArray procedure**

**Figure 2. Evaluation of EUROArray Lactase/Fructose Intolerance Direct**

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