K ras Proto Oncogenes

- **DPD deficiency**

General:

Dihydropyrimidine dehydrogenase deficiency (DPD deficiency) is an autosomal recessive metabolic disorder in which there is absent or significantly decreased activity of dihydropyrimidine dehydrogenase, an enzyme involved in the metabolism of uracil and thymine.

Individuals with this condition may develop life-threatening intoxication following exposure to 5-fluorouracil (5-FU), a chemotherapy drug that is used in the treatment of cancer. Beside 5-FU, a widely prescribed oral fluoropyrimidine capecitabine (Xeloda) might put DPD-deficient patients at risk, experiencing severe or lethal intoxication as well.

Indication:	5-FU therapy
Material:	10 ml EDTA blood
Preanalytics:	Blood shipment at ambient temperature (Do not freeze!)
TAT:	2 weeks*
Method:	PCR
Units:	U/l
Ref.- range:	wildtype, heterozygous, homozygous

- **UGT1A1 promoter polymorphism**

General:

This gene encodes a UDP-glucuronosyltransferase, an enzyme of the glucuronidation pathway that transforms small lipophilic molecules, such as steroids, bilirubin, hormones, and drugs, into water-soluble, excretable metabolites. This gene is part of a complex locus that encodes several UDPglucuronosyltransferases. The locus includes thirteen unique alternate first exons followed by four common exons. Four of the alternate first exons are considered pseudogenes. Each of the remaining nine 5' exons may be spliced to the four common exons, resulting in nine proteins with different N-termini and identical C-termini. Each first exon encodes the substrate binding site, and is regulated by its own promoter. Mutations in this gene result in Crigler-Najjar syndromes types I and II and in Gilbert syndrome.

Indication:	risk of Meulengracht disease (Gilbert syndrome), risk of toxicity after therapy with Irinotecan
Material:	10 ml EDTA blood peripheral
Preanalytics:	Blood shipment at ambient temperature (Do not freeze!)
TAT:	2 weeks*
Method:	PCR
Ref.- range:	wildtype, heterozygous, homozygous

- **c-Kit receptor (A502Y503Ins; exon 9)**

General:

KIT is a 145-kDa transmembrane glycoprotein and a member of the receptor tyrosine kinase subclass III family that includes receptors for platelet-derived growth factor (PDGF), macrophage-colony stimulating factor, and flt3. KIT is normally expressed by hematopoietic progenitor cells, mast cells, germ cells, interstitial cells, and gastrointestinal stromal tumors.

Mutations in KIT exons 9, 11, 13, and 14 were identified in gastrointestinal stromal tumors (GISTs) with the majority of changes involving the juxtamembrane region of KIT. Molecular modeling indicates that mutations in this region result in disruption of the KIT autoinhibited conformation, and lead to gain-of-function activation of the kinase.

GISTs are characterized by expressing a gain-of-function mutation in KIT. Imatinib mesylate, a tyrosine kinase inhibitor, has activity against GISTs that contain oncogenic mutations of KIT.

Indication:	therapy plan of GIST
Material:	tissue
Preanalytics:	One block paraffin embedded tumor tissue (7 x 7 mm) or six 7 µm sections of paraffin embedded tumor tissue on non-coated slides. If possible please use buffered formalin for fixing the tumor tissue specimen (pH 7.1 – 7.6), shipment at ambient temperature.
TAT:	3 weeks*
Method:	PCR
Ref.- range:	wildtype, mutated

- **c-Kit receptor (exons 9, 11, 13, 14, 17)**

General:

see above

Indication:	see above
Material:	tissue
Preanalytics:	One block paraffin embedded tumor tissue (7 x 7 mm) or six 7 µm sections of paraffin embedded tumor tissue on non-coated slides. If possible please use buffered formalin for fixing the tumor tissue specimen (pH 7.1 – 7.6), shipment at ambient temperature.
TAT:	3 weeks*
Method:	sequencing
Ref.- range:	wildtype, mutated

- **Platelet-Derived Growth Factor Receptor, PDGF-R (exons 12,14,18)**

General:

The platelet-derived growth factor binds to the protein tyrosine kinase receptors PDGF receptor-α and -β. These two receptor isoforms dimerize upon binding the PDGF dimer, leading to three possible receptor combinations, namely -αα, -ββ and -αβ. The extracellular region of the receptor consists of five immunoglobulin-like domains while the intracellular part is a tyrosine kinase domain.

Indication:	see above, c-Kit receptor (exons 9, 11, 13, 14, 17)
Material:	tissue
Preanalytics:	One block paraffin embedded tumor tissue (7 x 7 mm) or six 7 µm sections of paraffin embedded tumor tissue on non-coated slides. If possible please use buffered formalin for fixing the tumor tissue specimen (pH 7.1 – 7.6), shipment at ambient temperature.
TAT:	4 weeks*
Method:	sequencing
Ref.- range:	wildtype, mutated

- **Epidermal Growth Factor Receptor, EGFR (exons 18, 19, 20, 21)**

General:

Epidermal growth factor or EGF is a growth factor that plays an important role in the regulation of cell growth, proliferation, and differentiation by binding to its receptor EGFR. Human EGF is a 6045Da protein with 53 amino acid residues and three intramolecular disulfide bonds.

EGF acts by binding with high affinity to epidermal growth factor receptor (EGFR) on the cell surface and stimulating the intrinsic protein-tyrosine kinase activity of the receptor. The tyrosine kinase activity, in turn, initiates a signal transduction cascade that results in a variety of biochemical changes within the cell - a rise in intracellular calcium levels, increased glycolysis and protein synthesis, and increases in the expression of certain genes including the gene for EGFR - that ultimately lead to DNA synthesis and cell proliferation.

Mutations that lead to EGFR overexpression (known as upregulation) or overactivity have been associated with a number of cancers, including lung cancer and glioblastoma multiforme. In this latter case a more or less specific mutation of EGFR, called EGFRvIII is often observed. Mutations, amplifications or misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers.

Mutations involving EGFR could lead to its constant activation which could result in uncontrolled cell division – a predisposition for cancer. Consequently, mutations of EGFR have been identified in several types of cancer, and it is the target of an expanding class of anticancer therapies.

Indication:	therapy plan with Gefitinib (Iressa) or Erlotinib (Tarceva) for the non small cell lung cancer
Material:	tissue
Preanalytics:	One block paraffin embedded tumor tissue (7 x 7 mm) or six 7 µm sections of paraffin embedded tumor tissue on non-coated slides. If possible please use buffered formalin for fixing the tumor tissue specimen (pH 7.1 – 7.6), shipment at ambient temperature.
TAT:	4 weeks*
Method:	sequencing
Ref.- range:	wildtype, mutated

- **K-ras Proto-Oncogenes (exon 2, codons 12 and 13)**

General:

Ras is a family of genes encoding small GTPases that are involved in cellular signal transduction. Activation of Ras signaling causes cell growth, differentiation and survival. Ras is the prototypical member of the Ras superfamily of proteins, which are all related in structure and regulate diverse cell behaviors.

Since Ras communicates signals from outside the cell to the nucleus, mutations in ras genes can permanently activate it and cause inappropriate transmission inside the cell even in the absence of extracellular signals. Because these signals result in cell growth and division, dysregulated Ras signaling can ultimately lead to oncogenesis and cancer. Activating mutations in Ras are found in 20-25% of all human tumors and up to 90% in specific tumor types.

Indication:	therapy plan with Gefitinib (Iressa) or Erlotinib (Tarceva) for the non small cell lung cancer
Material:	tissue
Preanalytics:	One block paraffin embedded tumor tissue (7 x 7 mm) or six 7 µm sections of paraffin embedded tumor tissue on non-coated slides. If possible please use buffered formalin for fixing the tumor tissue specimen (pH 7.1 – 7.6), shipment at ambient temperature.
TAT:	2 weeks*
Method:	sequencing
Ref.- range:	wildtype, mutated

- **MSI**

General:

Microsatellites are repeated sequences of DNA. Although the length of these microsatellites is highly variable from person to person, each individual has microsatellites of a set length. These repeated sequences are common, and normal. The most common microsatellite in humans is a dinucleotide repeat of CA, which occurs tens of thousands of times across the genome.

In cells with mutations in DNA repair genes, however, some of these sequences accumulate errors and become longer or shorter. The appearance of abnormally long or short microsatellites in an individual's DNA is referred to as microsatellite instability. Microsatellite instability (MSI) is a condition manifested by damaged DNA due to defects in the normal DNA repair process. Sections of DNA called microsatellites, which consist of a sequence of repeating units of 1-6 base pairs in length, become unstable and can shorten or lengthen. Microsatellites are also known as simple sequence repeats (SSRs).

MSI is a key factor in several cancers including colorectal, endometrial, ovarian and gastric cancers.

Indication:	therapy plan with Gefitinib (Iressa) or Erlotinib (Tarceva) for the non small cell lung cancer
Material:	tissue and 10 ml EDTA blood

Preanalytics:	One block paraffin embedded tumor tissue (7 x 7 mm) or six 7 µm sections of paraffin embedded tumor tissue on non-coated slides. If possible please use buffered formalin for fixing the tumor tissue specimen (pH 7.1 – 7.6), shipment at ambient temperature.
TAT:	3 weeks*
Method:	sequencing
Ref.- range:	wildtype, mutated

- **Karyotyping**

General:

the following parameters are available:

- Translocation (9;22)(BCR/ABL)
- P53
- PDGFRA (eosinophilia)
- Del(13)
- PDGFRB (eosinophilia)
- ATM gene
- Del 5q
- trisomy 12
- Del 7q
- Aberration 17p13

For other miscellaneous parameters please ask the laboratory

Indication:	therapy plan with Gefitinib (Iressa) or Erlotinib (Tarceva) for the non small cell lung cancer
Material:	preferred 5 ml heparin bone marrow (100 U heparin/ml KM), alternative 10 ml heparin blood peripheral or smear bone marrow (at >10 % of malignant cells).
Preanalytics:	bone marrow must be in the lab within 24 after drawing, shipment at ambient temperature (do not freeze!).
TAT:	3 weeks*
Method:	FISH

- **Sequencing (BRCA1/2, MLH, MSH2, MSH6, APC, MEN II, NF1)**

Indication: therapy plan with Gefitinib (Iressa) or Erlotinib (Tarceva) for the non small cell lung cancer

Material: tissue

Preanalytics: One block paraffin embedded tumor tissue (7 x 7 mm) or six 7 µm sections of paraffin embedded tumor tissue on non-coated slides. If possible please use buffered formalin for fixing the tumor tissue specimen (pH 7.1 – 7.6), shipment at ambient temperature.

TAT: 4 weeks*

Method: sequencing

Ref.- range: wildtype, mutated

- **The following tests are also available. For more information, please contact us directly.**

- Circulating tumor cells (mamma)
- Septin 9
- - BRAF (V600E)-mutation
- EML4-ALK fusion gene

For complete list of laboratory test offered at Freiburg Medical Laboratory, please visit http://www.fml-dubai.com/parameter-listings/