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Review

# Next generation diagnostic molecular pathology: Critical appraisal of quality assurance in Europe

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ABSTRACT

Tumor evaluation in pathology is increasingly based on a combination of traditional histopathology and molecular analysis. Due to the rapid development of new cancer treatments that specifically target aberrant proteins present in tumor cells, treatment decisions are increasingly based on the molecular features of the tumor. Not only the number of patients eligible for targeted precision medicine, but also the number of molecular targets per patient and tumor type is rising. Diagnostic molecular pathology, the discipline that determines the molecular aberrations present in tumors for diagnostic, prognostic or predictive purposes, is faced with true challenges. The laboratories have to meet the need of comprehensive molecular testing using only limited amount of tumor tissue, mostly fixed in formalin and embedded in paraffin (FFPE), in short turnaround time. Choices must be made for analytical methods that provide accurate, reliable and cost-effective results. Validation of the test procedures and results is essential. In addition, participation and good performance in internal (IQA) and external quality assurance (EQA) schemes is mandatory. In this review, we critically evaluate the validation procedure for comprehensive molecular tests as well as the organization of quality assurance and assessment of competence of diagnostic molecular pathology laboratories within Europe.


## 1. Molecular diagnostics in pathology

Routine molecular diagnostic determinations of tumor specimens in the pathology laboratory have been performed since the late 1990's and concerned mainly classification of tumors, clonality determinations and tests such as microsatellite instability analysis (MSI) to select patients for referral to clinical geneticists. New biological agents that target specific

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molecular alterations or act to block activated pathways present in individual tumors have become available and enable treatment decisions based on the molecular features of the malignancy. This precision medicine has rapidly gained access to daily practice and it has become a challenge for molecular biologists and pathologists to provide relevant information on the predictive markers in the shortest timeframe possible.

In this review we will highlight aspects on choice and validation of comprehensive molecular assays including assays using next generation sequencing (NGS) technology, and on internal and external quality assurance of molecular tests in Europe. Before the challenges for the molecular pathology and validation and quality assurance issues will be discussed, we will first give a brief overview of the different molecular applications.

### 1.1. *Overview of different molecular applications in pathology*

The identification of mutations or chromosomal rearrangements that are characteristic for disease entities can assist the pathologist in the differential diagnosis of disease entities. For example, fusion transcripts are seen in the majority of sarcomas and can be, in the right pathological and clinical context, helpful as highly specific molecular diagnostic markers with significant impact on the classification of the tumor (Bovee and Hogendoorn, 2010; Demicco, 2013). Likewise, clonality assessment of the highly polymorphic immunoglobulin and T-cell receptor gene rearrangements is an important tool in the diagnostics of lymphomas. The clonality results should be interpreted with knowledge of the guidelines and the pathological and clinical context (van Krieken et al., 2007; Groenen et al., 2012; Langerak et al., 2012). Analysis of microsatellite instability (MSI) as a hallmark of Lynch syndrome-associated tumors is used to select patients suspected of having Lynch syndrome before referral to a clinical geneticist. Subsequent analysis of *MLH1* and *MSH2* promoter methylation is part of this diagnostics (van Lier et al., 2010), but also somatic mutation analyses of mismatch repair genes if no germ line mutation has been found in these patients after referral to a clinical geneticist (Mensenkamp et al., 2013; Geurts-Giele et al., 2014).

The identification of specific molecular characteristics may guide therapy. Genetic aberrations can discriminate if morphological similar or asynchronous tumors in one patient represent one or two entities or not e.g. whether a secondary tumor is indeed an independent tumor or a metastasis of a primary (van der Sijp et al., 2002; Blokx et al., 2007). Furthermore, analysis of the *MGMT* promoter methylation status in glioblastoma has become important for predicting outcome to treatment with temozolomide (Weller et al., 2013).

The observation that some genetic aberrations make tumor cells dependent on or "addicted to" a gene product or cellular pathway has powered the development of drugs that specifically target these aberrations allowing treatment based on the genetic makeup of a tumor, also called precision medicine (Weinstein, 2002). At present there are several genetic changes leading to targetable proteins. Examples are overexpression of ErbB2 (HER2) due to *ERBB2* amplifications in e.g. breast cancer, and treatment with trastuzumab (Herceptin) (Piccart-Gebhart et al., 2005) and activating *KIT* and *PDGFRA* mutations in gastrointestinal stromal tumors (GIST) as targets for the tyrosine kinase inhibitors (TKI) (Joensuu et al., 2001; Lasota and Miettinen, 2008). More recently, high volume screening for *EGFR* and *KRAS* mutations and *ALK*, *ROS1* and *RET* rearrangements in non-small cell lung cancer, *KRAS* and *NRAS* mutations in colon cancer and *BRAF*, *NRAS* and *KIT* mutations in metastasized melanomas has become an essential part of daily molecular pathology diagnostics to select patients for targeted treatment options (Chapman et al., 2011; Douillard et al., 2013; Lindeman et al., 2013).

## 2. Challenges for molecular diagnostic tests in a pathology laboratory

The efforts of The Cancer Genome Atlas (TCGA) initiative have led to a still growing body of information on acquired somatic genomic changes in different cancer genomes (Cancer Genome Atlas Research, 2008). Due to the fast increase of available targeted therapies as well, the TCGA efforts rapidly result in a growing demand for routine molecular tumor diagnostics and screening for actionable mutations in a wide variety of tumor types. To offer patients the best treatment options for a certain tumor, diagnostic tests should be reliable, reproducible, of sufficient high sensitivity, and able to investigate all potential targets with the constrains of limited amount of tissue, time and budget. These criteria for comprehensive molecular testing require permanent development of new assays, awareness of their potentials and drawbacks, continuous quality assessment to improve testing of diagnostic tissues, consciousness of budget and costs and clinical demands such as turnaround time. Apart from these issues there are tissue- and technological challenges as well.

### 2.1. *Tissue challenges*

There are many challenges typically for molecular pathology diagnostics of solid tumors. The vast majority of the DNA to be analyzed is retrieved from routine formalin-fixed, paraffin-embedded (FFPE) tissue, which leads to suboptimal DNA quality for the required assays due to fixation and histoprocessing procedures (see also Groenen et al., 2011). For the design of the molecular test it should be taken into account that suboptimal DNA samples (isolated from routine FFPE tissues) allow only amplification of small-sized PCR-amplicons (100–200 bp). In addition, the DNA is isolated from (dissected) tissue fragments composed of mixed populations of normal and neoplastic cells reducing the mutant allele frequency, and frequently only a limited amount of (biopsied) tumor tissue is available containing a low percentage of neoplastic cells, therefore the test developed must be able to accurately detect low levels of mutations.

### 2.2. *Technological challenges: a multitude of different molecular tests*

To detect the various genomic DNA alterations in the tumor cells including point mutations, large insertions and

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