FLI guidelines for marketing authorisation of PCR diagnostic test kits (PCR-kit, PCR- and extraction kit)

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Commercial test systems for the specific detection of nucleic acids of pathogens by polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR) or other methods of nucleic acid amplification and detection (e.g. NASBA) are to be registered, tested and approved like all other diagnostic test kits (e.g. ELISA) by the Friedrich-Loeffler-Institut (licensing authority Insel Riems) before being distributed and used in Germany (section 11 sub-section 2 Animal Health Act).
Within this procedure the producer's documentation and its accordance with the formal requirements is verified, experimental testing of PCR test systems is made in an adequate laboratory (e.g. national reference laboratory). Regular batch testing is made for approved test systems.

For admission to the marketing authorisation procedure PCR diagnostic systems must meet the following general requirements:

1. Clear identification of PCR diagnostic test kits as **"PCR kit"** only (all essential reagents exclusively for the PCR reaction are included in the kit and are tested; the extraction procedure corresponds to a standard procedure and is not part of the diagnostic test kit), or as **"PCR- and extraction kit"** (the extraction procedure is part of the test system, all essential reagents for PCR reaction and for extraction are part of the kit. The package insert is to be modified correspondingly.

2. The PCR diagnostic test kit includes at least one **positive control** and, if necessary, also negative controls are included. Additionally the diagnostic test kit includes at least one control, which shows, that the samples to be tested do not inhibit PCR (inhibition control). An appropriate extraction control is advisable.

3. **Type and quality of the samples to be used** are indicated in the package insert. The marketing authorisation is limited to these samples. In case of pathogens to be detected in various species, validation data for each species must be presented. The marketing authorisation is limited to the species of animal, for which the validation data had been presented.

4. With these samples the test is sufficiently **tested, standardized and validated**. A sound documentation must be presented. Validation tests by independent external institutions are advisable.

5. **Analytic and diagnostic specificity and sensitivity** of PCR diagnostic test kits are to be indicated and proved by a sufficient quantity of data, if necessary after consulting the responsible laboratory. The marketing authorisation application must be accompanied by an appropriate collection of validation data.

    a. **Analytic Sensitivity**: detection of a theoretic analytic detection limit on the basis of Plasmid DNA or in vitro synthesized RNA (e.g. with a defined number of copies) or a given number of pathogens, using the respective standards of each FLI laboratory or to follow specific instructions.
    b. **Analytic specificity**: Exclusion of genetically related pathogens. Specifications as to species or specificity of type must be verified presenting appropriate documentation. By a data based comparison of sequences (gene bank) sequence information of primer and probes used are to be tested and documented for cross-reactivity.
    c. **Diagnostic sensitivity and specificity**: At least data for 150 different positive samples (also with weak positive results, no replicates of few reference strains) as

well as data for at least 150 negative samples should be presented. If the diagnostic test kit aims at the detection of a broad range of pathogens (e.g. all influenza A viruses or all pestiviruses) a representative data profile should be presented (e.g. influenza A: sufficiently broad basis of representatives of all 16 actual HA subtypes). The diagnostic specificity of negative samples of the relevant species should also be tested.

d. **Range of the test**: In case of quantifying test systems the DNA/RNA standard range of each matrix, in which a reliable and stable quantification is possible, should be indicated.

e. **Reproducibility**: The comparability of results of one PCR run with the following PCR runs should be adequately documented.

6. Instruments, which are necessary for this particular PCR test kit and which are not available in the examination laboratory (e.g. real-time PCR cycler) must be placed at disposal free of charge during the market authorisation procedure and the batch testing.

7. For the marketing authorisation procedure a sufficient quantity of PCR diagnostic test kits must be available. Test kits for at least 250 single reactions have to be provided.

8. After the marketing authorisation is granted batch testing of PCR diagnostic test kits is also made by the FLI. For this purpose, kits of each corresponding batch must be available for the licensing office, and material for approximately 150 single reactions have to be provided.