Validation of a new line probe assay for the amplification and simultaneous detection of 21 Italian CFTR mutations.


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Introduction
The performance of INNO-LiPA CFTR Italian Regional assay for the detection of 21 Italian CFTR mutations and their wild-type sequences was evaluated in-house.

The aim of this study was to evaluate the diagnostic sensitivity and accuracy of the INNO-LiPA CFTR Italian Regional and INNO-LiPA CFTR Italian Amplification.

The CFTR gene has numerous mutations (>1000) and functionally important polymorphisms (>200). In Italy, a high allelic heterogeneity and variable frequency of causative mutations has been observed. In order to reach appreciable levels of detection rates (> 80%) for most Italian regions, a panel detecting 21 important Italian mutations was developed to supplement the INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn Update assays.

INNO-LiPA CFTR Italian Regional is a line probe assay based on the reverse hybridization principle. In order to detect the 21 mutations, one multiplex amplification (PCR) of 15 regions of the CFTR gene is required. The distribution in Italy of mutations that can be detected with the INNO-LiPA CFTR Italian Regional is shown in Figure 1. The layout of the strip is shown in Figure 2. The detection rate using INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn Update in combination with INNO-LiPA CFTR Italian Regional is shown in Table 1.

Methods
Study design

• Internal validation.
  • A total of 85 anonymous DNA samples originating from different countries were tested. These samples were selected on the basis of their mutation profile, which had been previously identified by one or a combination of alternative established DNA identification methods.
  • All DNA analyzed during the evaluation was extracted in-house.

Diagnostic sensitivity

• Diagnostic sensitivity in this evaluation was 97.7% (83/85; 95% CI [94.4%; 100%]) after initial testing and 98.8% (84/85; 95% CI [93.6%; 100%]) after a repeat testing of two samples for which amplification failed. However, further testing of the sample that failed twice at different DNA concentrations (range 0.36 µg to 1.08 µg in the PCR mix) yielded an interpretable LiPA result at each concentration.

• The aim of this study was to evaluate the diagnostic sensitivity and accuracy of the INNO-LiPA CFTR Italian Regional.

Table 1. Detection rate using INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn Update in combination with INNO-LiPA CFTR Italian Regional.

Results internal evaluation

Amplification

• The 85 samples were amplified with Primer Solution AMP CFTR Italian.

• DNA concentrations used were in the range of 0.07 to 0.2 µg/µL.

• HeiStarTag (Qiagen) was used.

• A PE9700 thermal cycler (Perkin Elmer) was used.

Hybridization

• The results were interpreted visually and a genotype was documented for each sample.

Figure 1. Italian distribution of mutations detected with the INNO-LiPA CFTR Italian Regional.

Figure 2. Strip layout.

Table 1. Detection rate using INNO-LiPA CFTR19 and INNO-LiPA CFTR17+Tn Update in combination with INNO-LiPA CFTR Italian Regional.

Results internal verification

• A total of 91 different samples (additional to the 84 samples mentioned above) were tested: 52 whole blood, 10 buccal brushes, 20 dried blood spots, and 9 Coriell samples.

• Mutations exhibited in these samples enabled testing of all mutations on the strip, and each mutation was tested at least once.

• DNA concentration ranged from 0.01 to 0.62 µg/µL.

• For buccal brushes and dried blood spots the extraction procedure followed that described in the product insert.

• No cross-reactivity was observed with 621+1G T (five samples), 1898+1G T (three samples), 1898+1G C (one sample), R334W (three samples), R347P (six samples), R1062C (one sample), R1162X (six samples) and S549R A = C (one sample).

• All 71 samples for which a reference result was available gave correct genotypes.

• Precision: 24 samples were analyzed in duplicate, manually as well as on Auto-LiPA, on three different membrane batches, performed by five different persons.

• A proficiency panel consisting of three samples with known reactivity was tested in-house by two different persons. A 100% agreement was observed.

Conclusion

• INNO-LiPA CFTR Italian Regional was successfully validated in-house on 85 samples.

• A total of 85 anonymous whole blood samples was amplified, 84 of which where analyzed with the regional strip.

• Accuracy was 100% (84/84) after discrepancy testing of 3 samples.

• INNO-LiPA CFTR Italian Regional is a reliable and accurate test for the detection of 21 cystic fibrosis-related mutations in the CFTR gene region.

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