DEVELOPMENT AND VALIDATION OF A NEW TUBERCULOSIS REAL TIME PCR KIT

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ABSTRACT

Bovine tuberculosis (TB) is a chronic disease of animals caused primarily by Mycobacterium bovis. The disease causes general state of illness, coughing and eventually death. It can affect cattle, but also wild animals and is an important threat to threatening public health. In this context, monitoring of these bacteria calls for diagnosis with reliable, sensitive and specific tools.

Applied Biosystems™ VetMAX™ M. tuberculosis Complex kit (MTBC), a new molecular tool, allows the simultaneous detection of Mycobacteria belonging to Mycobacterium tuberculosis complex (MTBC) and an internal control. To demonstrate the kit’s performance, verification studies were carried out on cattle and wild animals, such as cervids, wild boars and badgers.

41 strains including 22 non MTBC Mycobacteria were tested to evaluate the specificity of the assay. More than 750 negatives and 350 positives field samples (lymph nodes and surrounding tissues) were tested in collaboration with partner labs to evaluate diagnostic sensitivity and specificity in respect to their country requirements.

RESULTS

The kits fulfills all the validation criteria for PCR characteristics and complete method required by the French standard (NF U47-600-2) « Requirements and recommendations for the development and validation of qRT-PCR in Animal Health ». The specificity evaluated on different strains showed no cross-reactions with closely related pathogens or Mycobacteria not belonging to the MTBC. This kit had an efficiency close to 100 % and its PCR limit of detection is 16 copies per PCR (95% confidence interval). The experimental LOD is 1 or 2 US per microliter of spiked matrix according to purification test used. Tests results on field samples showed an "almost perfect agreement" with other method tested in each study. The diagnostic sensitivity was evaluated at 97.5 % [95.1-98.9 %] and diagnostic specificity at 99.4 % [96.6-99.9 %].

INTRODUCTION

Bovine tuberculosis (TB) is a chronic disease of animals caused primarily by Mycobacterium bovis. The disease causes general state of illness, coughing and eventually death. It can affect cattle, but also wild animals and is an important threat to threatening public health. In this context, monitoring of these bacteria calls for diagnosis with reliable, sensitive and specific tools.

MATERIALS AND METHODS

VetMAX M. tuberculosis Complex kit is a TaqMan® ready-to-use real-time PCR assay based on the simultaneous detection of Mycobacteria belonging to Mycobacterium tuberculosis Complex (MTBC) and an exogenous Internal Positive Control (IPC).

For the development of a reliable, sensitive and specific rPCR system, a large panel of Mycobacteria belonging or not to the MTBC, and other closed pathogens were tested. This product fulfills all the validation criteria of PCR characteristics and complete method, as required by the NF U47-600-2 standard.

The isolation of DNA from field samples carried out on bovine and wild animals, such as cervids, wild boars and badgers, was performed with MagMAX™ Universal isolation kit. More than 750 negative samples and 350 positive samples (lymph nodes and surrounding tissues) were collected by different partners and tested to evaluate diagnostic specificity and sensitivity, in respect to their country requirements.

The in vitro diagnostic performance of the VetMAX M. tuberculosis Complex kit was evaluated on a panel of 22 Mycobacteria not belonging to the MTBC and 19 other pathogens close to M. tuberculosis complex, either because they are preferably found in the same ecological niches, phylogenetically close, or because they have the same clinical symptoms in target species. None of the strains tested were detected.

The specificity evaluated on different strains showed no cross-reactions with closely related pathogens or Mycobacteria not belonging to the MTBC. This kit had an efficiency close to 100 % and its PCR limit of detection is 16 copies per PCR (95% confidence interval). The experimental LOD is 1 or 2 US per microliter of spiked matrix according to purification test used. Tests results on field samples showed an "almost perfect agreement" with other method tested in each study. The diagnostic sensitivity was evaluated at 97.5 % [95.1-98.9 %] and diagnostic specificity at 99.4 % [96.6-99.9 %].

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CONCLUSIONS

VetMAX M. tuberculosis Complex kit is a real-time PCR kit allowing the simultaneous detection of Mycobacterium tuberculosis complex (MTBC) and an exogenous positive control in lymph nodes and surrounding tissue samples.

ACKNOWLEDGEMENTS

• French Veterinary Lab – Dordogne (24)
• French Veterinary Lab – Côte-d’Or (21)
• French Veterinary Lab – Pyrénées-Atlantiques (64)

TRADEMARKS/LICENSES

• Applied Biosystems™ VetMAX™ M. tuberculosis Complex kit
• MagMAX™ Universal Isolation kit (Cat. No. MV384)

REFERENCES

• NF U47-600-2 « Animal Health analysis methods – PCR Part 2: Requirements and recommendations for the development and validation of qRT-PCR in Animal Health »

0.998; Eff=99.14%