



Improving laboratory diagnostic efficiency of epizootic diseases in Belgium



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Introduction

Belgium and other European neighbouring countries faced several **emerging and re-emerging diseases** as well as zoonotic diseases over the last decade. However, it has been noticed that during these episodes that adequate laboratory **diagnostic capabilities** were the clue to rapidly control and eradicate these diseases. An updated crisis scenario book, as it is implemented in the Belgian national laboratory (NRL), has showed all its usefulness during these situations.

Belgium plays a key role in international animal trade and making it very sensitive to diseases introduction via different pathways e.g. trade of live animals or animal products from around the world or via wildlife (translocation). It is therefore **crucial to react quickly** to these diseases, to establish appropriate contingency plans and to develop reliable diagnostic tests. Moreover, only few publications regarding the increase of diagnostic capacities are available in the scientific literature.

Materials and methods

Based on 29 selected diseases (Bianchini J., personal communication), a mapping of existing techniques was created. Published techniques based on a **literature review** of the OIE reference manuals and on publications available in PubMed and CAB abstract were consulted. An overview of **assays available** and which would be used in different scenarios in national and international laboratories was established. Finally, kits provided by the main commercial manufacturers/suppliers were obtained by an additional specific survey. If gaps were detected international laboratories were contacted in order to create future partnerships. Assays used in different **scenarios** (risk importation, early detection, spread and freedom of disease) were summarized in order to establish and map existing techniques. All data were gathered online in **decision trees** using Cmap® software, which allows a clear view of existing techniques and would facilitate decisions making by the authority in these different situations

Results and perspectives

Using LimeSurvey® web application a **large survey** in Belgium and abroad has been completed to implement a specific database and to determine laboratory capacities in Europe. Using **four scenario analyses** (risk of importation, early detection, spread and freedom of disease) diagnostic flow charts and questions, similar than those collected in the literature review, concerning kits characteristics (e.g. sensibility & specificity), as well as additional questions regarding laboratory capacities, the experience and the degree of accreditation were asked. Also bilateral partnerships (techniques and/or collaboration protocols) between Belgian NRL and other European institutions will be developed.

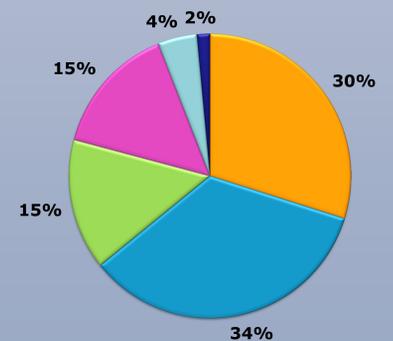
Frequency of assays used in routine in the Belgian national and regional laboratories

- National laboratory + 2 regional laboratories
- National laboratory + 1 regional laboratory
- National laboratory
- Regional laboratory
- No Belgian laboratory



Sum of the different assays used in the national and regional laboratories to detect the 29 diseases

- Enzyme-linked immunosorbent assays
- Polymerase chain reaction
- Virus neutralization test
- Virus isolation
- Hemagglutination inhibition
- Maldi-Tof



Online generic Cmap® diagnostic decision trees

[Link to online resources \(OIE Terrestrial Manual or Code, E.U. Legislation\)](#)

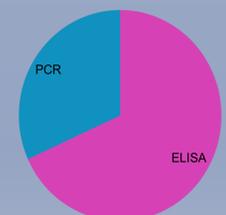
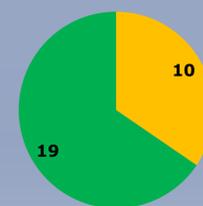
[OIE specific assays related to the scenarios](#)

[Notepad document](#)



Proportions of diseases for which a commercial assays have been developed and types of tests available

- No assay commercially available
- Assays available



These surveys have showed that **virus isolation** and **virus neutralization test** are sensitive and specific and are therefore often considered as gold standard confirmation tests. However, they are slow, laborious and require biosafety laboratory facilities. For many diseases, **enzyme-linked immunosorbent assays** (ELISA) are often used and commercially available. Although its rapidity, often good sensitivity and specificity, ELISA needs to be standardized and fit for purpose which requires training and experience. Molecular methods such as the **polymerase chain reaction** (PCR or mostly real-time PCR) are very specific and sensitive which give qualitative and/or quantitative result in a few hours but need adequate material (e.g. good extraction protocol). In addition, PCR's are very often sequence-dependent and thus sensitive to mutations and contaminations which can lead to false negative results. **Sequencing** offers promising solutions but is currently only used as confirmation for first suspicious samples and is still restrained for research purposes because of the price for the equipment, the samples preparation and the requirement for trained staff.

On the other hand, this project suggests the importance of implementing and keeping up-to-dated decision trees for (re-)emerging diseases. After a national survey, in addition to provide a clear overview of assays available, decision trees could permit, when analysing different scenarios, to determine lacks in diagnostic capacities or need in more reliable assays. Furthermore, comparing results from the literature review, the OIE recommended assays and the surveys including commercial companies and international laboratories, Cmap® figures could provide indications concerning the relevance of tests currently used in the Belgian laboratories but also to push forward new assays that should be implemented.

In conclusion, it was showed that Belgium is able to establish a diagnostic for 25 of the 29 of targeted diseases and, via international collaborations, has the diagnostic capacities to face all situations mentioned in the scenarios.

This work will help the Veterinary Authorities to take a faster, precise and well documented decision in case of an epidemic in Belgium.

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