Chapter 7

Molecular Approaches to Recognize Relevant and Emerging Infectious Diseases in Animals

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Abstract

Since the introduction of the first molecular tests, there has been a continuous effort to develop new and improved assays for rapid and efficient detection of infectious agents. This has been motivated by a need for improved sensitivity as well as results that can be easily communicated. The experiences and knowledge gained at the *World Organisation for Animal Health (OIE) Collaborating Centre for Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine, Uppsala, Sweden*, will here be used to provide an overview of the different molecular approaches that can be used to diagnose and identify relevant and emerging infectious diseases in animals.

Key words Infectious diseases, Pathogen detection, Molecular diagnosis, Transboundary animal diseases, Endemic diseases, Zoonoses, PCR, Isothermal amplification, Hybridization, Proximity ligation assay (PLA), Microarrays, Nanotechnology

1 Introduction

The increased occurrence and emergence of devastating infectious diseases, in both domestic and wildlife animal populations, are causing very serious socioeconomic losses at both global and regional levels. This increase has been attributed to several contributing factors, the most prominent being the accelerated movements of humans and animals due to increased globalization and international trade, the climatic changes, and the larger and larger populations kept together in animal husbandry and breeding. Some of these diseases, termed *transboundary animal diseases* (TADs), such as foot-and-mouth disease and classical swine fever, have a high capacity to spread very rapidly over countries and borders, having a devastating impact on animal productivity and trade, as well as causing other losses in the animal husbandries and in wildlife. Other diseases, such as anthrax, bovine tuberculosis, and rabies, have more *endemic* character,

establishing themselves in limited areas and showing slower tendency of spread. Considering their importance, many of these infectious diseases are listed by the World Organisation for Animal Health (OIE) as notifiable animal diseases, collectively referred to as OIE-listed diseases. The OIE is also determining and updating the *international animal disease status* on a regular basis. The current OIE-listed diseases and the latest disease status reports are available at the OIE website (www.oie.int).

Zoonoses, veterinary, and human public health. Of special importance among the animal infectious diseases are the ones that have the capacity to cross the species barriers and establish infections in a wider range of hosts including humans, causing *zoonotic infections*. It has been estimated that approximately 75 % of the new and emerging human infectious diseases over the past 10–20 years have been caused by pathogens originating from animals or from products of animal origin [1, 2]. Many of these diseases have the potential to spread through various means, over long distances, and to become global problems.

Accurate and rapid diagnosis. Considering the extremely high direct and indirect losses and other consequences caused by the TADs and the other infectious diseases, it is very important to develop and apply a wide range of diagnostic methods. These should preferably allow rapid detection and identification of the infectious agent(s), with high specificity and sensitivity, while still being affordable and readily available. When outbreaks do occur, rapid and accurate diagnosis is needed to screen susceptible populations and monitor the spread of the infectious pathogens, therefore helping with epidemiological investigation and implementation of necessary control measures, such as vaccination, stamping out, and quarantine restrictions, in order to prevent further spread.

Collection of clinical samples and sample preparation. Identification of the relevant groups of animals, showing clinical signs or at stages of infection when the presence of infectious agents is likely to be sufficiently high, and correct sampling are the first two crucial steps in the diagnostic process. The next steps of great importance are the sample preparation procedures, such as cleanup and target enrichment, which are performed in order to reduce possible contaminants and retain concentrated materials from the target agents, most commonly nucleic acid and/or proteins, for further analysis. If any of these steps are not properly considered and carried out, all diagnostic methods, even the most powerful and sensitive, will be unable to detect and identify the infectious agents, and this is leading to false diagnosis, which could have very serious consequences.

The OIE Collaborating Centre (OIE CC) for Biotechnology-Based Diagnosis of Infectious Diseases in Veterinary Medicine. Since the authors' institutes in Uppsala, Sweden, are well-recognized centers

of excellence in molecular diagnostics, the OIE has granted them the mandate to work together as its only collaborating center focused on biotechnology-based diagnostics (www.sva.se/en/About-SVA/OIE-Collaborating-Centre). In this chapter, the experiences and knowledge gained at the OIE CC will be used to provide an overview of the molecular approaches capable of recognizing relevant and emerging infectious diseases in animals.

Detection and identification of the infectious agents. The diagnostic laboratories can apply two basic ways for a proper diagnosis: (a) *direct detection and identification* and (b) *indirect detection and identification* methods. *Direct detection and identification* means that the infectious agents and/or their components, such as nucleic acids or proteins, are detected in the collected samples. Commonly used *classical diagnostic methods* for direct detection include identification of microorganisms by culture techniques and immuno-fluorescence, and the most widely applied *molecular diagnostic methods* are the various assays of nucleic acid hybridization, e.g., polymerase chain reaction (PCR) and isothermal amplification methods, such as the loop-mediated isothermal amplification (LAMP), among others. When running *indirect diagnosis and identification*, the immune responses of the host are investigated, looking for antibodies against various infectious agents, which indicate the occurrence of the infections in the hosts. In this chapter we focus on *direct diagnosis*, with special regard to *molecular diagnostic methods*, as well as some considerations regarding the interpretation, understanding, and communication of the diagnostic results.

2 PCR-Based Approaches

Molecular approaches become increasingly important in infectious disease diagnostics and, with the exception of isolation by culturing, may supersede all other direct detection methods. The main reasons are that a unique signature of every microorganism is encoded in its genome, which in principle enables perfect specificity, and that various enzymatic mechanisms can be utilized to manipulate and amplify the genetic material, yielding an exquisite sensitivity of the molecular DNA-based assays. While bacteria have their genome encoded in the form of DNA, some viral genomes are composed of RNA, and an initial reverse transcription step is therefore required before further manipulations and amplification can be carried out. Enzymes typically utilized are polymerases, reverse transcriptases, ligases, glycosylases, and nucleases. Of these, the polymerases require a pair of sequence-specific primers, which enables selective target amplification.

2.1 PCR Assays

PCR employs thermostable polymerases to enable amplification by continuous thermocycling and is currently the most commonly used method for amplification of genetic material [3]. The highly charged phosphodiester backbone of DNA makes the PCR product amenable to high-resolution visualization on agarose gel electrophoresis utilizing DNA-binding fluorescent dyes such as ethidium bromide. Electrophoresis both provides a means for detection by band visualization and enables at least a tentative verification of specificity by estimation of the amplicon length.

Shortly after the introduction of PCR, attempts were made to enhance sensitivity of detection of target nucleic acid sequences by running a second PCR assay targeting the internal region of the amplicon resulting from the first reaction, so-called nested PCR [4, 5]. The greater sensitivity has been attributed to both a dilution effect of any inhibitory compounds present in the sample, since only a minor fraction of the first reaction volume is used in the second reaction, and the fact that the primer-driven reaction is run twice, using four specific primers, rather than two. An intermediate situation is obtained if one of the primers from the first reaction is retained in the second, which yields a semi-nested PCR format.

The drawback of using PCR, and in particular the nested PCR formats, is that conserved regions must exist on the genome, and this might be a serious problem for highly variable RNA viruses. Although more recently the convenient and less laborious real-time PCR methods have been developed (*see* below) and are mostly used today in clinical practice, nested PCR assays are still used due to their high sensitivity and robustness.

2.2 Real-Time PCR Assays

Gel-based PCR is a heterogeneous, relatively laborious, detection method. Furthermore, it only reflects the end point of the PCR and, for this reason, doesn't allow the determination of the initial quantity of the detected material, e.g., determination of the viral load. Since it lacks specific markers for the targeted amplicon, unspecific amplification yielding similar product sizes may lead to false positive detection. Nested PCR has the further disadvantage of being prone to cross-contaminations since reaction tubes with potentially very high quantities of target DNA are opened between the two reactions. Many of these drawbacks were solved by the advent of real-time PCR [6]. With this technique, the PCR product is monitored in the course of the reaction using DNA-binding moieties that alter their fluorescence upon binding to the amplified DNA. This allows a closed tube, homogeneous assay format, which reduces the risk for cross-contamination and also removes the laborious gel electrophoresis step. In addition, the cycle number where the fluorescence reaches a defined threshold level will depend on the initial quantity of target DNA or RNA (before reverse transcription).

Three main approaches have been taken to monitor fluorescence alteration in real time due to the buildup of the PCR product, which can be ordered according to the level of specificity the methods provide. The simplest method is to add a fluorescent dye to the PCR mixture with the property that the fluorescence intensity changes upon DNA binding. Typical dyes are asymmetric cyanine dyes, such as SYBR green or tiazole orange, that exhibit a fluorescence increase when bound to DNA [7, 8]. These types of real-time PCR have no better specificity than gel-based PCR, rather the opposite, since no information is provided about the product length. New possibilities are given by tethering the dye to one of the PCR primers that are constructed so that incorporation of the primer into the amplicon leads to an alteration of dye fluorescence. Several chemistries have been devised to this end, for example, scorpion primers [9], LUX primers [10], and Plexor primers [11]. Although in principle not providing a better specificity in regard to spurious amplification than the pure dye approach, fluorescent primers enable multiplexing by co-adding several primer pairs, each with a distinct fluorophore. The third approach includes addition of a third fluorescently labeled oligonucleotide, located between the primers, called a probe. The probe can also be labeled with a quencher (dual-labeled probe) but not always, e.g., not for the LightUp probes [7] or in the PriProET approach [12, 13]. Prominent examples of methods based on dual-labeled probes include TaqMan [14] and molecular beacons [15].

The signal that can be obtained from a probe-based real-time PCR experiment is often limited by the competing reannealing of the double-stranded PCR amplicon. Asymmetric PCR can be used to overcome this problem since it allows preferential amplification of one strand in a double-stranded DNA template. This is achieved by manipulating primer properties, most critically concentration, as well as other factors influencing primer melting temperature, such as length and nucleotide sequence. In the LATE-PCR method [16], asymmetric PCR has been combined with molecular beacons for readout to achieve a detection format that allows quantification from the end-point fluorescence. This format is suitable for simpler portable PCR instruments designed for detection in the field and has recently been commercialized by various companies.

The application of real-time PCR techniques and other methods in molecular diagnostics in veterinary medicine have recently been extensively reviewed [17, 18] and will be further discussed later in this section. To conclude this subsection, it is suitable to mention a recently developed method for the rapid molecular pathotyping of avian influenza [19] and Newcastle disease [20] viruses that combines several of the themes discussed here. This technique employs a three level semi-nested PCR format that utilize Plexor [11] fluorogenic primers as a detection mechanism. Furthermore, the assay format allows a highly multiplex interrogation

of the sample by using primers in two vastly different concentration regimes. Instead of, as hitherto has been the case, requiring nucleotide sequencing over the hemagglutinin and fusion protein genes of avian influenza and Newcastle disease viruses, a much faster diagnosis can be obtained by a simple PCR-based method. This method could even be implemented on field PCR instruments for rapid on-site diagnosis and thereby providing means for faster containment of disease outbreaks.

3   Isothermal Amplification

Isothermal amplification of nucleic acids is an alternative method to PCR. The reaction is performed at a constant temperature in simple devices, such as water baths or heating blocks, which eliminates the need for high-end equipment and system maintenance. It can be used to test for infections in regions where resources are limited and logistic chains are impossible, but a rapid answer is needed. Isothermal amplification normally takes about an hour or less to complete, providing a fast specimen-to-result diagnosis at the point of care (POC). To make the best use of isothermal amplification, a system should ideally integrate the upstream sample preparation and the downstream detection steps and be operated by personnel without extensive training. Several platforms utilizing isothermal technology are commercially available or close to market [21].

Recently, the field of isothermal amplification technologies has advanced dramatically, resulting in several different amplification systems. These have been summarized by Niemz et al. [21] and include transcription-mediated amplification (TMA) [22], helicase-dependent amplification system [23], loop-mediated isothermal amplification (LAMP) [24], and rolling-circle amplification [25]. Of those methods, LAMP has gained the greatest interest because of its high specificity, efficiency, and rapidity. By addition of a reverse transcriptase in the reaction, RNA targets can also be amplified and detected by LAMP, which is referred to as RT-LAMP. The LAMP utilizes four primers that bind to six distinct regions of the target DNA to specifically amplify a short region and is catalyzed by *Bst* DNA polymerase with strand-displacement activity [24]. Addition of loop primers may accelerate the reaction [26]. As of 8 February 2014, PubMed listed 990 publications with the search term "loop-mediated isothermal amplification." LAMP technology has been applied for the detection of viral pathogens such as classical swine fever virus [27] and foot-and-mouth disease virus (FMDV) [28], bacteria such as *Clostridium difficile* [29], and parasites such as malaria [30]. Commercial developments have progressed: a total of eight LAMP kits are approved in Japan for the detection of SARS coronavirus, *Mycobacterium tuberculosis* (TB), *Mycoplasma pneumoniae*, *Legionella* species, influenza A virus, H1

pdm 2009 influenza virus, H5 influenza virus, and human papilloma virus, as reviewed by Mori et al. [31]. Future development would need to consider simplification of sample preparations, reaction mix in a dried down formation and integration of all three steps in a compact, disposable, and inexpensive system.

4   Detection by Hybridization-Based Approaches

Identification and classification of bacteria and viruses using DNA hybridization-based approaches rely on the use of oligonucleotide probes that selectively bind to target sequences based on the degree of complementarity. This was early utilized in fluorescence in situ hybridization (FISH), which became a valuable tool for localization of infectious agents in clinical samples without cultivation [32]. However, to overcome limitations in multiplex capacity, sensitivity, and signal intensity, there has been an ongoing development of the initial approach. This has resulted in high-throughput methods such as DNA arrays but also interesting new hybridization-based methodologies combined with signal amplification, such as padlock probe (PLP) [33] and proximity ligation assay (PLA) [34]. PLP belongs to the methodologies of genomic partitioning where one specific region of the genome is massively replicated, and thereby detectable, even though it normally is masked by the presence of other genomes or in too low amount to be detected. PLA relies on the primary detection of antigens followed by oligonucleotide amplification and subsequent detection by fluorescent probes or by RT-PCR.

4.1   DNA Array Technologies

With the development of DNA macro- and microarray technologies, it became possible to detect and characterize a wide variety of bacteria and viruses through simultaneous hybridization against large numbers of DNA probes immobilized on a solid support [35, 36].

The probes represent known sequences that may serve as markers for identification and/or genotyping of bacterial strains, resistance genes, viruses, etc. These are commonly arranged in an ordered array of spots (or features), and hybridization with a labeled target, i.e., the sample to be investigated, will therefore result in a hybridization profile in which individual probe results also can be assessed. As the names imply, the main difference between macro- and microarrays is the number and size of spots on the support. Macroarrays typically have larger and fewer spots and have proven particularly effective for detecting smaller subsets of genes, such as genes involved in antibiotic resistance [37]. Microarrays can contain thousands, and even up to many *hundred thousands*, of spots with different oligonucleotide probes and have

successfully been used for detection and genotyping of bacterial and viral pathogens [38, 39]. The main advantages of microarray technology are high throughput, parallelism, miniaturization, and speed. However, microarrays are still considered to be an expensive technology and usually require large amounts of nucleic acid targets. Furthermore, unless it has been completely automated, the data analysis procedure might be time-consuming, and the results can be difficult to translate into information that is clearly communicable and decision supportive.

4.2 Genomic Partitioning

Genomic partitioning refers to the methodologies used for capture and enrichment of target regions. Within these methodologies, PLP has been used repeatedly for genotyping, localization, and array-based diagnostics. The earliest version of PLP consisted of two oligonucleotide probes of 20 nt connected by a linker region of 40 nt [33]. As the probes hybridize towards the target, the construct is ligated into a circular detector that can be replicated isothermally by Phi29 polymerase [40]. The detection can then be performed through incorporation of fluorophore tagged nucleotides. The PLP concept was further expanded with the introduction of the molecular inversion probe (MIP) technology. Where PLP leaves no gap after hybridization to the target region, MIP aims at leaving a single nucleotide gap. This gap is then filled in by addition of a single type of nucleotide into the assay. This approach enables substitutions on nucleotide level to be detected using just four reactions easily set up in a normal lab environment. It also provides a possibility of highly multiplexed designs of assays [41, 42]. Building on the same principle as PLP and MIP, the connector inversion probe (CIPer) technology extends the gap up to a few hundred nucleotides. Using DNA polymerase to fill the gap generates a product that can be sequenced, revealing the content of the target region [43]. Applications of PLP methodology and its derivatives for infectious diseases in animals include detection of all hemagglutinin and neuraminidase subtypes of AIV [44], as well as multiplex detection of FMDV, swine vesicular disease virus (SVDV), and vesicular stomatitis virus (VSV) [45]. In addition, by designing different probes for the genomic and replicative form of the virus, it is possible to not only detect a virus but also localize it in relation to the host cells and perform semiquantitative analysis of the amount of replicative viruses, as demonstrated with porcine circovirus type 2 [46].

4.3 Proximity Ligation Assay

Although PLA is designed for detection of protein interactions and localization using antibodies for target recognition, hybridization events are required to generate a detectable signal [34]. Two sets of antibodies are designed: one targets the protein/s of interest and the other target the first set. The antibodies in the second set carry short oligonucleotide strands that can hybridize with special

connector oligonucleotides and thereby enable the formation of circular DNA constructs. These are amplified and detected by PCR and fluorescent probes. The methodology combines dual antibody specificity with the signal amplification power of DNA amplification to produce a versatile and sensitive method for detection of very low amounts of targets. It also enables in situ localization studies of protein targets within cells [47]. Furthermore, PLA requires little to no sample preparation, making it ideally suitable for screening of massive amounts of samples, and can be used with a solid support to capture antigens for detection, similar to ELISA. The use of a solid support may also facilitate the removal of contaminants from the sample, thereby enabling PCR-based detection without the problem of inhibition. By combining the solid-support approach with RT-PCR detection, great sensitivity was demonstrated in a study of avian influenza virus [48]. Other applications of PLA technology include detection of several viruses, among them FMDV, with detection levels close to those of RT-PCR and 100-fold more sensitive than ELISA [49], as well as localization of influenza virus proteins within cells [50].

5   Further Trends, New Tools in Molecular Diagnostics

In the development of new molecular diagnostic methods, there has been a continuous effort to enable efficient and rapid detection of infectious agents from ever-smaller volumes of complex fluids without the need for a skilled operator. As a result, microfluidic analysis systems and nanotechnology-based detection devices have gained increased popularity, as previously reviewed [51, 52]. These systems and devices have been employed to construct a *wide range of integrated tools*, capable of semiautomated complex diagnostic procedures, which also allow rapid, portable field-based testing [53].

5.1   Microfluidic Analysis Systems

Several sequential laboratory procedures are usually required to detect infectious agents in clinical samples, such as concentration, lysis, extraction, purification, amplification, and product detection. Recent progress in microfluidic technology has allowed multiple procedures to be incorporated in sequence for one-step sensing or in parallel for high-throughput screening [54, 55]. These integrated systems with use in molecular diagnostics are more commonly known as biochip or lab-on-a-chip (LOC) devices. Since they usually consist of fluid channels and sensing chambers with dimensions of a few to hundreds of microns, very small amounts of sample can be analyzed, requiring only low consumption of reagents. The use of materials that can be easily functionalized, such as glass and plastic, allows the inner surfaces to be coated with different capture and sensing agents, e.g., antibodies and nucleic acids. Although this makes microfluidic analysis systems versatile

tools for pathogen detection, the main application involves systems based on the recognition of target nucleic acids.

As discussed above, the detection of target nucleic acid from a pathogenic microorganism or virus can be achieved either by direct probing or by first introducing an amplification step. *Amplification-based detection* usually gives higher sensitivity and has successfully been implemented on microfluidic chips using both PCR and alternative amplification methods, such as nucleic acid sequence-based amplification (NASBA) [56]. However, regardless of amplification method, it is first necessary to concentrate and lyse the sample material to extract and purify the nucleic acid. As described in the review by Heo et al. [57], a variety of alternative solutions have been developed to perform these sequential steps on a microfluidic chip. Popular strategies for sample concentration include magnetic beads [58] and dielectrophoresis [59]. The lysis of enriched samples can then be achieved by various methods, such as thermal energy, optothermal energy, mechanical force, and chemicals [60]. For the purification of extracted nucleic acid, packed silica beads, microfabricated structures, and magnetic beads have all proved to be useful solutions [61]. After amplification, the detection of products is most commonly performed with fluorescence or electrochemical methods, which easily can be miniaturized. A classification into three categories was suggested for microfluidic chips that use amplification-based detection by Mairhofer et al. [52]. These categories included microfluidic chips with (1) a stationary chamber as nano-/picoliter reservoir for conventional thermocycling, (2) a continuous flow where the sample is moved between individual temperature zones at different locations for cycling, and (3) a droplet-based system where each amplicon is individually amplified within a water-in-oil droplet. Examples of fully implemented amplification-based systems include devices for detection of different viruses, such as dengue virus and enteroviruses [62], as well as various bacteria, most notably *Bacillus anthracis* [63, 64].

5.2 Nanotechnology-Based Detection

Nanotechnology has extended the limits of molecular diagnostics to the nanoscale (one-billionth of a meter), allowing diagnostic assays to take advantage of the unique electrical, magnetic, luminescent, and catalytic properties of nanomaterials. This has contributed to the development of innovative assays that provide rapid detection of infectious agents with improved sensitivity and limit of detection (LOD) [65]. Because of the small scale, nanotechnology can also be used to create high-density arrays of sensors for high-throughput detection without increased sample requirements. Moreover, the use of sensitive nanoscale sensors has the potential to eliminate the need for sample preparation and target amplification, making it possible to construct assays for direct detection in opaque media, like blood and milk [51].

There has also been a special focus to develop affordable nanotechnology-based devices that provide fast and reliable results in simple and user-friendly formats for use even in rural areas of developing nations [66].

Most sensor systems for diagnostic use are comprised of two components, one receptor for target recognition by specific binding and one transducer that convert receptor readings into a signal that can be measured, such as an electronic or optical signal [67]. Nanoscale sensors are usually comprised of biological recognition elements coupled to different nanomaterials for signal transduction and detection. These nanomaterials include noble metal nanoparticles, nanobarcodes, quantum dots, and magnetic nanoparticles [68–71]. Nanowires and nanotubes can also be coated with biological recognition elements to be used as nanosensors, and binding events are measured as a change in their electrical conductance [72]. Another example is silicon-based cantilever sensors functionalized with biomolecules such as DNA for target recognition. These sensors are often combined into high-density arrays for high-throughput screening [73]. Applications of nanoscale sensors for detection of infectious agents include multiplex detection of both viruses and bacteria [74, 75].

6   Summary and Final Remarks

As detailed above, numerous molecular methods have been developed for the detection and characterization of infectious agents in the field of veterinary and human medicine. Among them, PCR has been the most commonly used technology. When considering the development of new technologies, a general trend can be observed towards robust and affordable automatic systems that also integrate sample preparation steps for rapid and highly sensitive multiplex detection of an easily enlarged panel of pathogens, both bacterial and viral. Although few, if any, of the novel systems have successfully incorporated all of these properties, they still represent important technological advancements towards more sensitive and efficient detection. Even so, only a limited number have so far been developed into commercial diagnostic kits, and only a few molecular tests are offered by veterinary diagnostic laboratories. The full potential and impact of molecular diagnosis is therefore yet to be realized. A possible explanation may be that most new published assays are only analytical validated and not properly evaluated in accordance with the appropriate criteria for field validation. New molecular tests might also not comply with current accreditation standards.

For the interpretation and understanding of the diagnostic results, it is very important to put the molecular diagnostic methods in the context of the complex scenario of infectious diseases, i.e., to follow not only the technical rules and procedures of the

molecular methods but also simultaneously acquire sufficient medical understanding in order to gain a more complete picture. A "PCR positive result" by itself, without analyzing the complex scenario, can be unreliable, even misleading, and may cause serious problems for the veterinary and human medical health authorities during implementation of eradication programs. To avoid such problems and to provide a reliable diagnosis, it is important to obtain a complete medical understanding of the disease scenario. Reliable diagnoses can be reached, of course, even on the basis of single PCR assays, if they are raising the right questions and the results are professionally communicated. On the other hand, there are many cases of infectious diseases where the diagnosis is more reliable if a range of various methods, both direct and indirect approaches, are applied simultaneously. It should therefore be emphasized that in certain cases, the simultaneous application of novel molecular diagnostic methods and classical approaches, such as isolation by culturing, is required for a fully reliable diagnosis.

Another important, but often neglected, aspect is the communication of diagnostic results. The successful control and eradication of infectious diseases is strongly accelerated and enhanced if the diagnostic laboratories are able to communicate the results rapidly and properly towards the practitioners and the health authorities. It is very important to pay sufficient attention even to this task, because a rapid and clear two-way communication between the laboratories and the practitioners, as well as the decision-making authorities, is essential in order to assure the success of the control and eradication programs.

The authors' institutes that constitute the OIE CC have been early developers and adopters of new diagnostic technologies and approaches, from the first PCR-based assays until today's plethora of various molecular methods, closely following and participating in the ongoing effort to develop improved tests. More recently, this has resulted in the adaptation and evaluation of PLP and PLA for detection of veterinary important pathogens, as well as a new PCR-based multiplex platform for molecular pathotyping of viruses, among other contributions. The OIE CC has had an important role in the development of novel molecular diagnostic methods, in international standardization and validation, as well as in international dissemination of results, outreach, and training. These activities are done with the support of the OIE, our home institutes SVA and SLU, and in collaboration with large international and national consortia of various EU projects, such as LAB-ON-SITE, ASFRISK, CSFV_goDIVA, AniBioThreat, RAPIDIA-FIELD, and Epi-SEQ. National grant agencies are also supporting this work, such as the Formas BioBridges Strong Research Environment project No. 2011-1692, which is supporting the diagnostic developments for the improved diagnosis of a wide range of poultry pathogens, many of which have zoonotic features, in the spirit of the One World, One Health concept.

Acknowledgments

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