PCR APPLICATIONS FOR DIAGNOSIS IN ANIMAL HEALTH

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Viral infectious diseases are a constant risk for livestock production due to the economic and sanitary costs produced by their entrance into a country. Once the causative agent is identified, specific strict sanitary measures must be rapidly implemented to avoid its spread, and to control and eradicate the disease. Classical laboratory diagnosis of viral diseases includes both direct and indirect tools, virus isolation being the "gold-standard" technique to confirm the presence of an etiological agent. Around 15 years ago, the merging of the PCR completely transformed the diagnostic pathway in the National Reference Laboratories and soon became an essential tool. Many benefits prompted its incorporation into routine lab work, high sensitivity, superior to virus isolation, being of most value as allows the early detection of pathogens even before the evidence of clinical signs in infected animals (table 1). The high specificity, the analysis of a great number of any kind of clinical samples within hours, and the chance to test several pathogens in the same reaction (multiplex assays), made PCR a basic tool for screening diagnostic step (figure 1).

The introduction of the real-time PCR changed the workflow once again in lab diagnosis, by reducing analysis time and contamination risk, and increasing the sensitivity and applications in diagnosis. Moreover, real-time PCR accompanied by the appearance of robots in the market for sample preparation and nucleic acid extraction steps, made real a completely automated analysis procedure for a high throughput application (figure 1).

Now, PCR has a wide range of main applications in Animal Health. It is extensively used in surveillance, control and eradication programs of the major viral diseases affecting livestock at national and international levels (figures 1, 2, 3, 5). PCR is a valuable tool in the prevention of new disease entrance, the control of biological products, or the evaluation of vaccines efficacy (figure 4). Generic PCR assays can be developed for detection of new viruses within a genus/family or to determine the subtype/serotype of the identified virus (table 2). Also, PCR methods can be designed to be used as DIVA ("differentiating infected from vaccinated animals") tests, discriminating vaccine strains from field circulating viruses, which can be of great significance when using live attenuated vaccines. Finally, PCR is the starting point in molecular characterization and epidemiology studies of circulating viruses, essential for tracing the origin and evolution of a disease outbreak.

More recently, portable PCR machines and other simple low-cost PCR-based techniques are being launched for on-site application, meaning the analysis of samples can be performed in regional labs or even in the farm/slaughter-house with basic equipment by non-specialized personnel. Due to many viral animal diseases are prone to very rapid spread and the identification of the involved virus is urgently required, these new tools may become useful first line pen-side tests in a short time.

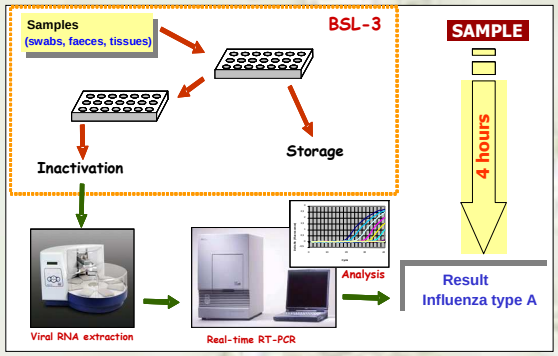
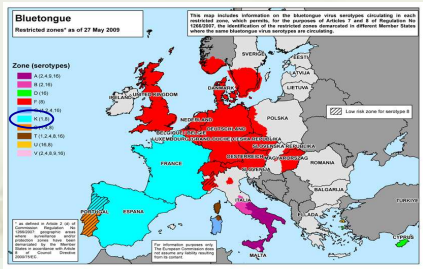
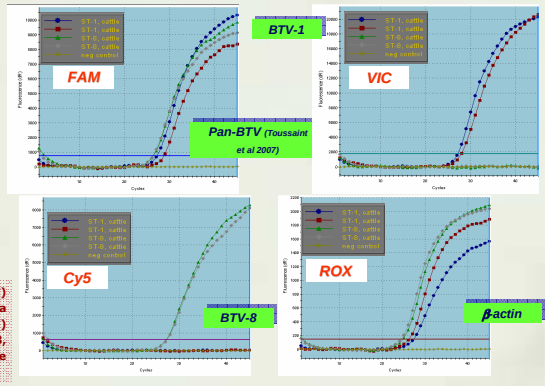


Figure 1: PCR workflow followed for Avian Influenza surveillance program at Spanish Reference Laboratory (LCV, Algete, Madrid). High throughput PCR analysis of clinical samples is performed totally automated and diagnostic results are ready within four hours since the reception of samples into the lab. (BSL-3: Biosecurity level-3)





Figures 2 and 3: Due to the complex Bluetongue (BT) epidemiological situation occurring in Spain during last years, a fourplex real-time RT-PCR method for generic BT virus (BTV) detection and specific identification of BTV serotypes 1 and 8, including an internal control, was developed for use in routine diagnosis of the disease.

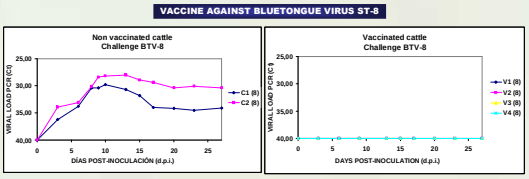
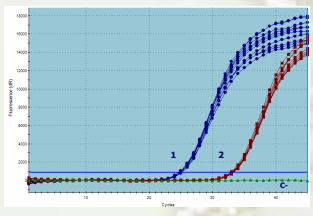


Figure 4: Current eradication strategies against Bluetongue (BT) are based mainly in the use of mass vaccination of susceptible animals employing serotype specific inactivated vaccines. Vaccines efficacy experiments carried out in bovine and ovine species include performing viraemia studies by real-time RT-PCR analysis of blood samples.



dpi	Sample	UPL#A (Ct)
0	Blood	No Ct
	Serum	No Ct
1	Blood	33.25
	Serum	No Ct
2	Blood	35.82
	Serum	No Ct
3	Blood	26.18
	Serum	32.60
4	Blood	21.25
	Serum	24.38

Figure 5 and table 1: A highly sensitive, specific and repeatable PCR method has been developed for the improved detection of African swine fever virus (ASFV) using commercial Universal Probe Library (UPL, Roche Applied Science). The assay allows the molecular diagnosis of this devastating porcine viral disease within just two hours, being able to detect the virus presence even before the appearance of clinical signs in the infected animals (table 1 shows the viraemia study of an ASFV experimentally infected pig, dpi: day post-inoculation). The use of commercial probes facilitates the acquisition of ready-to-use reagents at a comparable lower cost and makes easy the implementation and standardisation of new high throughput PCR systems in the lab.

Sample num	Isolate Identification	Recommended EU M-gene real-time PCR	H1 rRT-PCR	H2 rRT-PCR	H3 rRT-PCR	H4 rRT-PCR	H6 rRT-PCR	H8 rRT-PCR	H10 rRT-PCR	H11 rRT-PCR	H16 rRT-PCR
1	A/Duck/Alb/35/76 H1N1	24,17	**21,47**	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
2	A/Dk/Germ/1215/73 H2N3	28,36	NoCt	**26,47**	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
3	A/Turkey/Eng/69 H3N2	24,58	NoCt	NoCt	**20,55**	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
4	A/Dk/Czech/56 H4N6	24,49	NoCt	NoCt	NoCt	**22,81**	NoCt	NoCt	NoCt	NoCt	NoCt
5	A/Ost/Den/72420/96 H5N2	28,77	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
6	A/Ost/RSA/946/98 H6N8	22,46	NoCt	NoCt	NoCt	NoCt	**17,82**	NoCt	NoCt	NoCt	NoCt
7	A/Tky/Eng/647/77 H7N7	29,7	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
8	A/Turk/Ont/6118/68 H8N4	24,93	NoCt	NoCt	NoCt	NoCt	NoCt	**22,12**	NoCt	NoCt	NoCt
9	A/Tky/Wise/1/66 H9N2	24,89	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
10	A/RSA/Eg/Goose/238/98 H10N9	27,14	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	**26,91**	NoCt	NoCt
11	A/Dk/Eng/Eng/56 H11N6	26,41	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	**25,44**	NoCt
12	A/Duck/Alb/60/76 H12N5	23,17	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
13	A/Gull/Maryland/704/77H13N6	29,57	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
14	A/Mall/Guri/244/82 H14N6	30,18	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
15	A/SheerW/WA/2576/79 H15N6	25,72	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt
16	A/Gull/Dk/68110/02 H16N3	26,44	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	NoCt	**22,87**

Table 2: Avian influenza virus (AIV) subtyping can be performed directly by HA subtype specific real-time RT-PCR assays. Sixteen representative isolates belonging to all AIV HA subtypes were analysed in parallel by using generic EU AI M rRT-PCR assay, and by each novel developed HA-subtype RT-PCR method. Results are shown as Ct values.

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