THE USE OF NON-STRUCTURAL PROTEINS

TO DIFFERENTIATE BETWEEN VACCINATED AND INFECTED ANIMALS

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Summary: *This document provides a short review on the use of NSP tests to differentiate between vaccinated and infected animals; particularly focusing on the use of these tests to support FMD control programmes in regions that are endemic for foot and mouth disease (FMD) or sporadically impacted by the disease such as the 20 countries within the OIE Regional Commission for the Middle East.*

Keywords: *differentiating infected from vaccinated animals (DIVA) – foot and mouth disease virus (FMDV) – Middle East – non-structural proteins – vaccine.*

Serological tests are widely used to monitor the immune status of animals exposed to foot-and-mouth disease virus (FMDV) or FMDV vaccines. One particular application of these assays is to identify animals in a vaccinated herd that have been infected with FMDV. This so called DIVA (**d**ifferentiating **i**nfected from **v**accinated **a**nimals) principle exploits differences in the antibody (humoral) responses generated in vaccinated animals compared to those animals naturally infected with FMDV (whether or not they have been vaccinated).

High-quality FMDV vaccines are purified to contain structural protein (SP) viral capsid components from which most of the viral non-structural proteins (NSP) have been removed. In contrast, during natural infection with FMDV, NSP of the virus are expressed that elicit a corresponding immune response that can be detected using diagnostic approaches (Fig. 1).

During the replication cycle of FMDV, 8 different NSPs (as well as additional precursors) are generated which are potential serological targets for diagnostic assays [6]. Comparative studies using recombinant Lb, 2C, 3A, 3D, and 3ABC FMDV NSPs have highlighted considerable variability in the responses; however, following exposure to infection, vaccinated animals show an antibody response to NSP, particularly 3AB, 3ABC [10, 11], 2B [2, 9] and/or 3C, 2C, and occasionally 3A [10, 11].

Today, there are a number of commercially available tests, and in-house assays that detect NSP-specific antibody responses including 3ABC, 2B, 2C, 3B, 3B2, 3D. The strength of the NSP-specific antibody responses in individual vaccinated animals can vary according to the extent of virus replication. Therefore, when the comparative performance of five 3ABC assays and one 3B tests were evaluated [4], the ability of these tests to detect vaccinated animals that have been subsequently exposed to FMDV varied considerably (from 38% to 74%), although these sensitivity values were higher when only carrier animals were included in the analysis (48% to 89%). The specificity of all these assays in vaccinated cattle exceeded 96% [4]. Tests that adopt a blocking (antigen-capture) ELISA format provide a generic approach to detect NSP-specific responses for all species that are susceptible to FMD.

This document provides a short review on the use of NSP tests to differentiate between vaccinated and infected animals; particularly focusing on the use of these tests to support FMD control programmes in regions that are endemic for FMD or sporadically impacted by the disease such as the 20 countries within the OIE Regional Commission for the Middle East.

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In FMD-free countries such as those in Europe and North America, NSP tests in enzyme-linked immunosorbent assay (ELISA) formats have been exploited to support control policies that follow the 'vaccinate-to-live' concept, and are adopted into contingency plans for use in the event of FMD incursions [15, 17].

In contrast to SP tests such as the SPCE (solid-phase competition ELISA), LPBE (liquid-phase blocking ELISA) or VNT (virus neutralization test), NSP ELISAs are not serotype specific and can therefore be used as generic screening tools. Therefore, in addition to their use to detect virus circulation in vaccinated livestock populations, these tests are also used more generally for serological investigation, even when emergency vaccination is not practiced. However, the design of sampling surveys is critical when these assays are used to support national programmes to attain the OIE status of FMD-free without vaccination (i.e. to identify animals in which virus is circulating or has established persistent infections), since random surveys are not always effective at detecting rare events. In these circumstances, survey design is most effective if it accommodates epidemiological risk factors to direct sampling of animals [15].

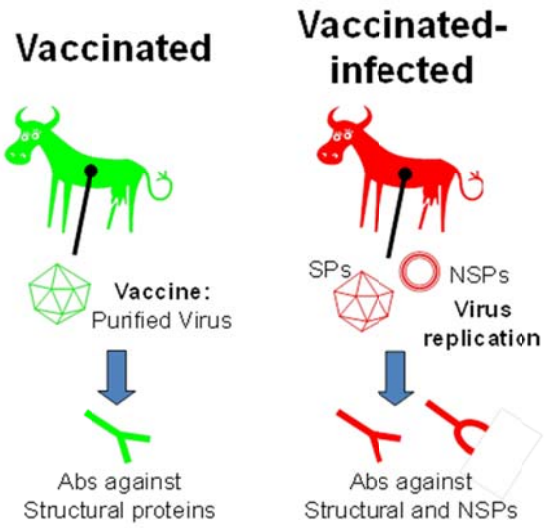

Fig. 1
The principle of using non-structural proteins (NSPs) tests to differentiate between vaccinated and infected animals.
Both structural (SP) and NSP antigens induce the production of antibodies in infected animals. In contrast, vaccinated animals that have not been exposed to replicating virus will only develop antibodies to the viral capsid (SP) antigens.

In endemic settings, NSP tests can be used to support sero-surveillance exercises that assess the prevalence of infection in livestock [18, 19, 20, 21] and wildlife [7], especially where the results for SP tests might be complicated by the presence of vaccine-induced antibodies. Following infection, NSP sero-conversion usually takes 7-14 days after which these antibodies can be detected in serum for months, or even years, depending upon the amount of virus replication [8, 12, 16]. In this scenario, it is important that only high-quality vaccines (that have been purified to remove contaminating NSPs) are deployed into the study region.

Even so, study designs usually focus on younger animals (<18 months of age), since repeated vaccination, even with high quality vaccines, can generate positive signals in the NSP ELISAs that may provide a false indication of FMDV infection [8]. As countries move towards OIE FMD-freedom (with vaccination), NSP surveys play an important role to confirm the absence of FMDV circulation in livestock populations [5]. Since these exercises involve the testing of relatively large numbers of sera, it is usually important to adopt a layered testing approach to accommodate the inherent performance of the NSP assays [4] and the expected number of false positives.

Tests with high diagnostic sensitivity (such as a 3ABC ELISA) are normally used to screen the sera, and positive results are confirmed using a second NSP antibody assay at least equivalent sensitivity and specificity [4, 15]. Furthermore, to rule out the false-positives, epidemiological investigations and analysis of probang samples by real-time RT-PCR may be recommended [15, 16]. In this context, SP testing could be explored but would require a detailed knowledge of typical responses against the vaccine to identify unexpectedly high titres associated with infection. For these studies, it is important

to know the design prevalence of the study (such as to detect 5% prevalence with 95% confidence) since this will impact upon the interpretation of data. Rather than employing random sampling, the adoption of a risk-based sampling strategy may be more effective to recognize rare events.

When used for ruminants, a limitation of these tests is that they are unable to distinguish between convalescent and carrier animals [3, 14]. Therefore, while these NSP antibody tests can be useful to estimate the degree of virus circulation in a population, whether these positive animals can contribute to the epidemiology of FMD is not well understood.

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