

## Short Communication

## Correlates of Protection for Influenza Vaccines

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## Abstract

The immunogenicity of a vaccine is estimated in terms of its capacity to induce an antibody and/or cell-mediated immune response in recipients. Studies have shown that antibodies against viral haemagglutinin are important correlates of protection.

Traditionally, the assessment of immunogenicity is based on two serological assays, the haemagglutination inhibition assay and single radial haemolysis. A haemagglutination inhibition titer of 40 is considered to be an immunological correlate of protection and is regarded as the best available parameter of protection against infection. Although the correlates of protection have been used for decades, they are still a matter of discussion.

**Keywords:** Correlates of protection; Influenza vaccines; Haemagglutination inhibition assay.

Influenza vaccines are the main means of implementing prophylaxis against seasonal and pandemic influenza viruses [1-3]. The immunogenicity of a vaccine is estimated in terms of its capacity to induce an antibody and/or cell-mediated immune response in recipients [4]. Studies have shown that antibodies against viral Haemagglutinin (HA) are important correlates of protection [5,6].

The European Committee for Medicinal Products for Human Use (CHMP) has revised its guidelines on influenza vaccines in order to provide a single guideline covering different aspects, such as the quality, regulatory, non-clinical and clinical aspects of the development of influenza vaccines (as of February, 2017) [7].

Traditionally, the assessment of immunogenicity is based on two serological assays, the Haemagglutination Inhibition Assay (HI) and Single Radial Haemolysis (SRH) [7,8]. The HI assay is currently the most widely used and detects antibodies capable of preventing agglutination between red blood cells and HA [9,10]. Despite its wide application, this assay has some limitations, including low sensitivity to B and avian strains, inadequacy in the evaluation of Live Attenuated Vaccines (LAIVs), a high degree of variability among laboratories and a lack of standardized protocols [10-17]. The HI antibody titer is expressed as the reciprocal of the highest serum dilution that shows complete inhibition of agglutination. An antibody titer of 40 is generally regarded as a protective threshold level, beyond which there is a 50% or greater reduction in the risk of infection in susceptible populations. An HI titer of 40 is considered to be an immunological correlate of protection and is regarded as the best available parameter of protection against infection, according to international regulatory guidelines for influenza vaccines [5,18,19]. However, two issues arise. First, how reliable is a threshold that was defined a long time ago and is based on

challenge studies in healthy adults with potentially attenuated viruses? Second, influenza vaccines are recommended not only for healthy adults but also for children, the elderly, pregnant women, anyone with high-risk conditions and those with chronic medical conditions; it is not known whether the HI titer of 40 is equally applicable in these groups. For instance, Black, et al. [20] provided evidence that an HI titer equal to or greater than 40 is not an appropriate correlate of protection in children under 6 years of age, as they need an HI titer of 110 to reach 50% protection and titres of 215, 330 and 629 to achieve protection levels of 70%, 80% and 90%, respectively. Other studies have suggested that cellular, rather than humoral, immunity correlates with protection in the elderly [8,21,22]. In addition, the HI assay seems to be inadequate for the evaluation of LAIVs, whether seasonal or pandemic, for which correlates of protection have not yet been established [9,14-16]. However, it is important to underline the fact that vaccines that display low immunogenicity in terms of HI antibodies may generate other types of immunity [3]. One of the most promising alternatives could be the immune response against the stalk of the HA, which seems to be able to induce broader protection. Immune correlates for this new generation of vaccines need to be investigated and established, as they would be different from the traditional assays [3,23].

The other serological assay is the SRH assay based on the passive haemolysis of red blood cells; the haemolysis mediated by complement and induced by the antibody-antigen complex produces easily identifiable "areas of haemolysis", which are proportional to the concentration of antibodies against influenza viruses present in serum samples. This assay detects antibodies against surface glycoprotein and internal antigens [24-26]. A haemolysis area of 25 mm<sup>2</sup> or greater is generally defined as a protective threshold level, beyond which there is a 50% or greater reduction in the probability of contracting influenza infection [27]. Neither the HI nor the SRH assays are standardized [7].

The Virus Neutralization (VN) assay has been officially included in the guidelines on influenza vaccines [7]. This technique is able to detect functional antibodies involved in the protection process that prevent the virus from entering or replicating in mammalian cells. The VN titer is expressed as the reciprocal of the serum dilution that shows at least 50% inhibition of cytopathic effect in mammalian cell cultures [9]. VN has proved to be more sensitive than HI. However, the complexity of the assay makes VN more susceptible to high inter-laboratory variability, due to the lack of common reference protocols and standardized parameters such as virus inoculations, incubation time and cell preparation [13,28]. As yet, there are no defined correlates of protection for the VN assay and, owing to the variability of the assay; a VN titer equivalent to an HI titer of 40 is highly specific for each antigen-laboratory combination [29]. Several studies have evaluated possible correlates of protection for the VN assay. With regard to the H5N1 virus and other influenza infections, some studies have defined a titer equal to or greater than 80 as an efficacy endpoint for avian influenza vaccines, while others have identified a seroprotection cut-off of 20 as suitable, on the basis of its correlation with an SRH area of 25 mm<sup>2</sup> [30-34]. In order to overcome the lack of standardized assays, the guidelines support the inclusion of adequately qualified laboratories, validated assays and in-house controls, unified protocols and standard

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reagents, including international standards, if available. In addition, cooperation among regulatory authorities, academia, public health institutions and manufacturers is aimed at standardizing the assays [35].

Guidelines on influenza vaccines have also included the possibility to evaluate the immune response against Neuraminidase (NA), the second glycoprotein of the viral surface, which is involved in viral release and spread from infected cells. The NA response contributes significantly to immune protection by reducing the severity and duration of infection and by curbing viral shedding and transmission [36,37]. However, the NA content is not standardized in the current influenza vaccines [8,21]. Assays for the evaluation of NA response have been designed and need to be further developed in order to define specific correlates of protection for NA [3,9].

Cell-Mediated Immunity (CMI) has been considered a promising alternative to the humoral response, especially in some groups, such as the elderly. Although the EMA guidelines encourage the evaluation of CMI, the correlates of protection based on antibody response are improper for this assessment.

Overall, it seems quite unrealistic to imagine that one single parameter could be used for different vaccine formulations and types, different age-groups and health status and different immune responses (humoral and cellular) to influenza vaccinations [9].

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