Technical note: Detection and differentiation of dengue virus in the context of dengue vaccine administration[1]

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Objective and scope

The main objective of this document is to provide technical recommendations for the detection and differentiation of wild-type and/or vaccine dengue virus (DENV) in people who have received the TAK-003[1] (Qdenga®) vaccine and who present symptoms compatible with a DENV infection (1) within 30 days after having received a dose of the vaccine (first or second dose). For other dengue vaccines that are of the same type (live attenuated virus vaccines), the same diagnostic recommendations apply. Likewise, this document will be reviewed and updated when new biologics, new clinical trials with additional evidence, or new diagnostic platforms are available and should be considered.

Background

Dengue virus (DENV, *Orthoflavivirus denguei* species, family *Flaviviridae*) is the etiologic agent of dengue disease. Although dengue is endemic and occurs in epidemic cycles, since 2023 there has been a marked and unusual global increase in the transmission of the four DENV serotypes (DENV-1 to DENV-4), including in previously dengue-free areas. In the Americas, as of epidemiological week 44 (EW44) of 2024, 12.479.437 suspected cases of dengue have been reported, representing an increase of 204% compared to the same period in 2023 and 381% compared to the average of the last 5 years (2). In this context, some countries have introduced the TAK-003 vaccine, which is administered in a two-dose schedule with an interval of three months between doses. This quadrivalent live attenuated vaccine contains four viral strains: one strain of DENV-2 and three chimeras of the other serotypes that were generated by replacing the envelope (E) and pre-membrane (prM) genes of DENV-2 with those of the wild-type dengue virus strains DENV-1, DENV-3, and DENV-4. The genes of the non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) and the capsid (C) remain constant for all the strains included in the vaccine and belong to the DENV-2 serotype.

In the phase 3 clinical trial, the TAK-003 vaccine showed efficacy in preventing dengue disease for all 4 serotypes in people with a previous history of DENV infection (seropositive before vaccination) (3). In people who were seronegative before vaccination, the vaccine showed efficacy for serotypes 1 and 2, but not for serotypes 3 and 4. In general, specific antibodies produced during infections constitute an important part of the immune response to neutralize pathogens; however, in the case of pathogens such as DENV, under certain conditions, the antibodies generated in an initial infection can potentiate new infections. The phase 3 clinical trial did not have sufficient statistical power to rule out the risk of vaccine-enhanced disease in people who were seronegative before

[1] Live attenuated vaccine developed by Takeda Pharmaceutical Company Limited. The mention of specific companies or products of certain manufacturers does not imply that they are endorsed or recommended by the Pan American Health Organization (PAHO). Likewise, there is no commercial relationship, nor conflicts of interest for the development of this technical document.

vaccination and who are exposed to wild-type DENV infection after vaccination. Vaccine-enhanced disease in a subpopulation of seronegative people is biologically plausible and cannot be ruled out. Finally, the reactivation of virulence of the vaccine virus is a hypothetical risk of the vaccine, although in the results of pre-clinical and clinical research, no cases of vaccinated people with dengue disease with the vaccine virus have been identified.

For these reasons, the Pan American Health Organization (PAHO) Technical Advisory Group (TAG) on Vaccine-Preventable Diseases notes that, if any Member State wishes to introduce the TAK-003 vaccine, this introduction should be carried out within a framework that allows for the evaluation and monitoring of the safety and effectiveness of the vaccine. In addition, communities and health professionals involved must be fully informed of the potential benefits and risks of vaccination (4).

One of the strategies used to monitor the safety of a vaccine is the surveillance of Events Presumably Attributable to Vaccination and Immunization (ESAVI). An ESAVI is defined as "any unfavorable and unintended health situation (sign, abnormal laboratory finding, symptom or disease) that occurs after vaccination or immunization and that does not necessarily have a causal relationship with the vaccination process or with the vaccine" (5). After vaccination with live attenuated vaccines, mild symptoms are commonly seen as indicative of immune system stimulation. However, it is theoretically possible that after vaccination a disease clinically compatible with dengue caused by one or more vaccine strains develops, which in places of DENV circulation could be confused with the disease caused by wild-type DENV.

For more details on monitoring vaccine safety, please refer to the safety guidance published by PAHO in the document "Regional Guidance for Dengue Vaccine Safety Surveillance - TAK-003".

Taking into account the current scenario of significant circulation of DENV in the Region of the Americas, and considering the available data on the efficacy of the TAK-003 vaccine and that the protection it confers is not immediate (it takes time to generate a protective immune response after having completed the two-dose schedule), a vaccinated individual could be infected with wild-type DENV a few days before or after vaccination (first or second dose). Given the genetic and antigenic similarity between vaccine strains and wild-type strains, it is important to consider laboratory methods for their detection and differentiation. It should be noted that this document does not address the diagnosis of a possible vaccine-enhanced disease in people who are seronegative before vaccination and exposed to wild-type DENV infection after vaccination.

Laboratory methods for the detection of viruses present in the TAK-003 vaccine

Laboratory confirmation of infection with a wild-type DENV strain is based on virological tests such as RNA detection by reverse transcription followed by real-time polymerase chain reaction (RT-qPCR), detection of NS1 antigen by ELISA, and in some cases viral isolation. In general, serological tests (detection of IgM and/or IgG by ELISA) are not considered confirmatory in a single sample (6). In principle, vaccine strains can be detected using the same assays.

RNA detection by RT-qPCR

Although **RT-qPCR** is a highly sensitive and specific method that could allow the detection of wild-type and/or vaccine DENV involved in an infection or ESAVI, there is still no standardized and validated assay that allows the differentiation between vaccine and wild-type DENV strains. There is evidence that the TAK-003 vaccine can cause viremia of up to 21 days, and in exceptional cases for up to 30 days; viremia has only been observed after the

application of the first dose of the vaccine (3, 7 – 9). For this reason, in patients who meet the definition of a dengue case and who have received a dose of the TAK-003 vaccine in the 30 days prior to the onset of symptoms, it is recommended to collect a serum sample for RT-qPCR. This sample should be collected up to 5 days after the onset of symptoms, taking into consideration that the shorter the time between the onset of symptoms and the collection of the sample, the greater the probability of detecting the viral RNA.

It is important to note that the detection of vaccine strains by currently available RT-qPCR assays should be carefully analyzed. Depending on the design of these assays, some vaccine strains, particularly chimeric strains of TAK-003 (DENV-1, DENV-3, and DENV-4) may not be detectable. For example, the detection of DENV-1 in the CDC DENV-1-4 Real Time RT-PCR Assay (10) is based on the identification of a region of the *NS5* gene of this serotype (nucleotides 8936 to 9047), a gene that is not present in the DENV-1 chimeric vaccine strain of TAK-003. For DENV-3 and DENV-4, the same assay is based on the identification of the prM region (nucleotides 701 to 775 and 884 to 973, respectively) that is present in the respective chimeric vaccine strains. Therefore, it is expected that this assay will detect DENV-2, DENV-3 and DENV-4 vaccine strains but not the DENV-1 vaccine strain. This analysis should be performed for each of the currently available RT-qPCR assays that can identify wild-type DENV, such as the CDC's Trioplex RT-PCR assay (11). Therefore, a positive RT-qPCR result would generically indicate the presence of wild-type virus and/or vaccine strains detectable by the specific assay being used. Furthermore, while a negative result would generally rule out the presence of a wild-type virus in a good quality sample taken within the first five days after symptom onset (6), this result does not rule out the presence of a vaccine strain that might not detectable by the assay or which viraemia might be below limit of detection of the assay.

Detection of NS1 protein by ELISA

Regarding **NS1 viral protein detection by ELISA,** a recent study conducted in a group of 351 single-dose vaccinated individuals showed that the NS1 antigen was not detected after vaccination with TAK-003 (12). However, since vaccine viruses may generate NS1 proteins during replication, it cannot be ruled out that this antigen may be detected depending on the levels of antigen produced and the sensitivity of the test used. In addition, this technique does not discriminate whether the DENV causing the infection is wild-type or vaccine-derived. Thus, a positive result would only indicate the presence of the virus and not differentiate whether it is wild-type and/or vaccine-derived DENV.

Serological tests (detection of antibodies)

The studies published so far do not describe in detail the kinetics of the antibodies generated by the first or second dose of the TAK-003 vaccine, but it is assumed that this kinetics is similar to that induced by a wild-type virus. Therefore, **serological assays** that detect specific IgM and IgG antibodies can't differentiate between a vaccine-induced and wild-type virus-induced immune responses. In addition, the difficulties commonly associated with the use of serology in confirming DENV infection (persistence of antibodies after an acute infection, cross-reactivity of antibodies produced by infections by different orthoflaviviruses, particularly for secondary infections (6)) also apply if such confirmation is required in patients vaccinated with the TAK-003 vaccine or any other live attenuated vaccine against DENV. Therefore, a positive IgM ELISA result does not discriminate between acute or recent infection with wild-type virus or with vaccine virus or other orthoflaviviruses.

Sequencing

In consideration of the above, the only technique available to date that would allow for the differentiation and characterization of wild-type vs. vaccine DENVs is **genomic sequencing**. Generic next-generation sequencing protocols can be used after verification that they allow sequencing of the strains present in the TAK-003 vaccine. If not, these protocols will need to be updated. It is also important to note that the bioinformatic analysis of the sequencing data obtained must take into account that in some cases both wild-type and vaccine virus sequences could be present in the sample.

Finally, it is important to consider the fact that the vaccine induces a lower viral load compared to wild-type DENV strains. Thus, it is possible that in mixed infections (wild-type and vaccine viruses) minority variants corresponding to the vaccine strain might not be identified when using next-generation sequencing protocols.

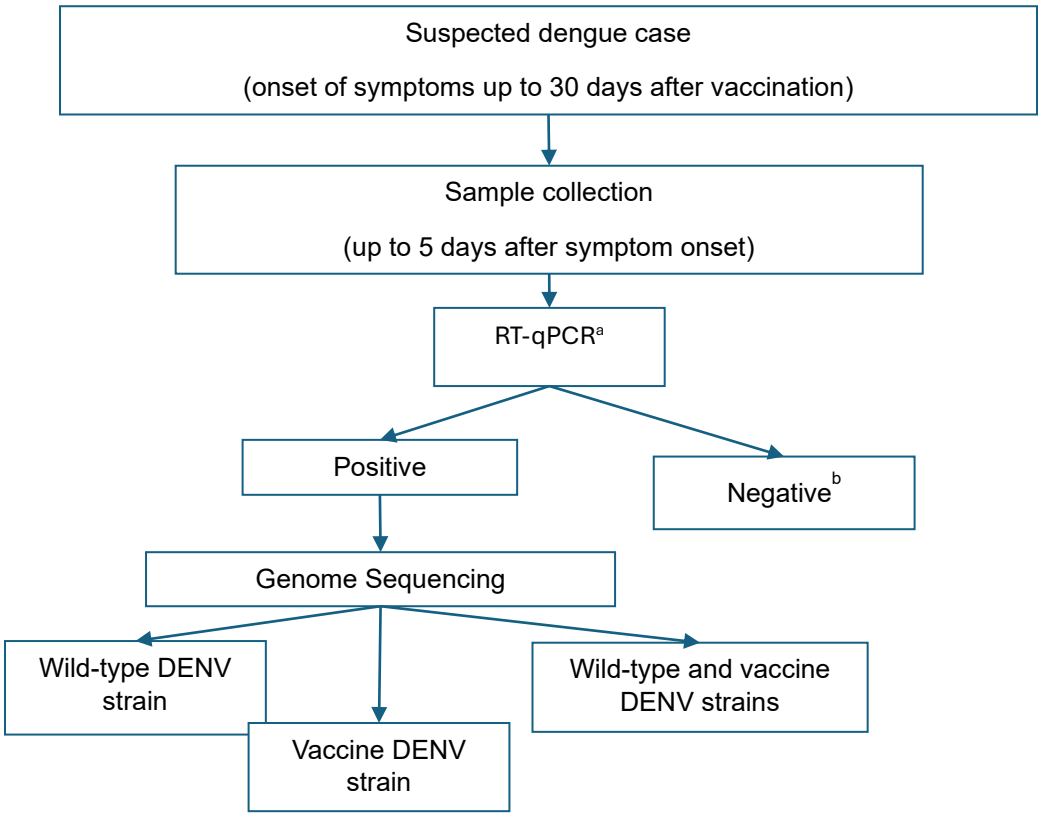
Some sequencing protocols using the Sanger methodology could also be used to differentiate between vaccine and wild-type DENVs; however, standardized and validated protocols for this purpose are not yet available.

Specific RT-qPCR assays

It is important to emphasize the need to develop and validate RT-qPCR assays that would allow for the differentiation between wild-type and vaccine DENV strains, like those available for yellow fever virus (e.g., 13). This type of tool will allow to quickly detect one or both DENVs (wild-type or vaccine) without the need to perform viral genome sequencing.

Diagnostic recommendations

The diagnostic recommendations apply to all people who meet the definition of a suspected dengue case[2] with onset of symptoms within 30 days after vaccination (first or second dose).



[a] RT-qPCR is the recommended technique during the acute phase of the disease and its sensitivity allows viral RNA to be detected for 5 days from the onset of symptoms or even more. If RT-qPCR is not available, detection of the NS1 antigen by ELISA may be considered, taking into account that its sensitivity is lower than RT-qPCR, and considering that a positive result should lead to sequencing while a negative result does not totally rule out the presence of vaccine virus.

[b] In general, a decrease in viremia is observed with time from the onset of symptoms, which may affect the sensitivity of molecular detection, particularly in samples collected from the fifth day after onset.

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[2] Definition of suspected case (1): A person who lives in or has traveled in the previous 14 days to areas with dengue transmission, and presents with acute fever that has typically lasted 2 to 7 days, and two or more of the following clinical manifestations: nausea or vomiting, exanthema, headache or retro-orbital pain, myalgia or arthralgia, petechiae or positive tourniquet test (+), leukopenia, with or without any warning sign or sign of severity.

Any child who resides in or has traveled in the previous 14 days to an area with dengue transmission that presents acute febrile symptoms, usually for 2 to 7 days, without an apparent focus, is also considered a suspected case.

Given the probability of replication of vaccine strains after 30 days after vaccination (first or second dose), suspected dengue cases identified after this period should be analyzed according to the general recommendation (6). However, in these cases, serology results should be interpreted in the context of recent vaccination as the duration of the antibodies generated by the vaccine is unknown.

Specimens

The recommended sample in suspected dengue cases is serum. In fatal cases, tissue samples should also be obtained. Tissue samples (liver, kidney, lung, lymph node, thymus, bone marrow, and brain) can be used both for genetic material detection (RT-qPCR) and viral antigen detection by immunohistochemistry. However, current immunohistochemistry techniques do not differentiate between vaccine and wild-type DENV antigens. The tissues can also be used for histopathological studies to better characterize the case.

Some recommendations for obtaining tissue samples can be reviewed in the ESAVI surveillance manual (5).

Recommendations for the transport of samples have been published previously (14).

It is recommended to maintain the cold chain during the transport and storage of samples selected for sequencing. These samples should not have undergone repeated freeze/thaw cycles, as this can alter sequencing results. It is important to consider the RT-qPCR Ct value to correctly select samples for sequencing: in general samples with Ct values ≤ 25 guarantee good quality sequencing, as long as the sample has been collected, transported and stored properly following the recommendations (14).

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