Bundesinstitut für Impfstoffe und biomedizinische Arzneimittel
Federal Institute for Vaccines and Biomedicines

Paul-Ehrlich-Institut

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Information for Healthcare Professionals

TESTING OF COVID-19 mRNA VACCINES

Methodology for Testing COVID-19 mRNA Vaccines for Alleged Contaminants

Due to a large number of inquiries from healthcare professionals, the Paul-Ehrlich-Institut would like to provide information on the current developments regarding alleged contaminants in vaccines. This information should also serve to inform both unsettled patients and those willing to vaccinate.

A large share of the data and studies on suspected contamination of COVID-19 mRNA vaccines circulating in the public are based on methodological deficiencies. There is also the issue of potentially improper storage of the vaccine doses tested. Experimental determinations, e.g. to test for residual third-party DNA in vaccine doses available on the market, must meet the following criteria in order to produce scientifically valid results:
(i) They must not be taken using samples from expired (expiration date exceeded) vaccine vials or from opened or improperly stored vaccine vials.
(ii) The methodology used to determine the amount of residual DNA must be demonstrably suitable and comprehensible – in particular, test interference should be ruled out by the presence of lipid nanoparticles in the vaccine vials (which cannot be guaranteed when tested on the final vaccine vial).
(iii) The method used must be validated to provide reliable and verifiable results.

In the frequently cited preprint publications by McKernan et al. (April 2023)[1] and Speicher et al. (October 2023)[2], there is a lack of sufficient information as to whether the aforementioned conditions have been met, as well as information on the comprehensibility of the chosen methodology. Method validation is essential to ensure that reliable and reproducible results are achieved at all times with the implementation of the method used, regardless of the person performing it, and that the method is suitable for its intended purpose. Manufacturers comply with the above-mentioned conditions for obtaining scientifically tenable measurement results in residual DNA determinations.

Part of the plasmid DNA serves as a template for the production of the COVID-19 mRNA vaccines. After transcribing the relevant DNA sequence into mRNA, the plasmid DNA is then comminuted by means of enzymatic digestion with DNase and depleted via the purification process to obtain the active substance (mRNA). However, a residual amount of plasmid DNA is present in small amounts that are considered harmless below a threshold specified in the marketing authorisation. To date, there is no evidence to suggest that any adverse events could be associated with residual DNA levels in authorised COVID-19 mRNA vaccines.

The Paul-Ehrlich-Institut would like to explicitly state that no DNA from cells of animal origin is used in the production of COVID-19 mRNA vaccines. Exclusively plasmid DNA of bacterial origin is used in the production process. Possible risks that could arise from residual animal cell DNA are a potential tumourigenicity due to the transmission of proto-oncogenes and potential DNA infectivity due to the transmission of completely functional viral genes. These risks are not present with DNA of bacterial origin. In this context, the WHO guideline "Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks" and the US FDA guideline "Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications" are not used for the production of mRNA vaccines. This is due to the fact that both guidelines explicitly refer to cells of animal origin, not to

[1] McKernan Kevin, Helbert Yvonne, Kane Liam T, McLaughlin Stephen (April 2023): Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose.
[2] Speicher David J, Rose Jessica, Gutschi L. Maria, Wiseman David M, McKernan Kevin (October 2023): DNA fragments detected in monovalent and bivalent Pfizer/BioNTech and Moderna modRNA COVID-19 vaccines from Ontario, Canada: Exploratory dose response relationship with serious adverse events.

bacterial cell substrates. Bacterial cells are expressly excluded from the guidelines.

Irrespective of this, the regulatory principle applies that as few contaminants as possible should be present in a vaccine and even theoretical risks should be reduced as far as possible. Therefore, very conservative limits for residual DNA have been set for the authorised COVID-19 mRNA vaccines and they may not be exceeded. Both residual bacterial genomic DNA and residual plasmid DNA are tested in the course of the production process. The fragmentation of plasmid DNA via DNase treatment of the mRNA, as it is done in the authorised COVID-19 mRNA vaccine products, provides additional safety, because even if complete and functional genes were contained, they would be almost completely degraded by DNase digestion during production and thus rendered harmless. This is because small DNA fragments are considered harmless as they cannot code for functional proteins (FDA Guidance for Industry (2010): "Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications").

The testing for residual DNA is not part of the official experimental OMCL (Official Medicines Control Laboratory) testing for batch release. Experimental OMCL testing of samples of each authorised vaccine batch includes the product-specific laboratory efficacy (potency) and safety parameters identified as relevant based on the evaluation of the vaccines in the authorisation process. The decision regarding the parameters to be reviewed is made in parallel with and based on the content of the benefit-risk assessment of each vaccine candidate as part of the authorisation process. This decision is the responsibility of the OCABR (Official Control Authority Batch Release) network and is based on a scientific consensus of the official experts. They identify and determine within an official procedure the product-specific critical test procedures, test parameters, and release criteria to be reviewed in the laboratory that are relevant to the efficacy and safety of an authorised vaccine product. The decision is evidence-based and scientifically substantiated as it is based on data and findings collected as part of the development process and reviewed in the authorisation process.

In addition to the experimental testing of the specified efficacy and safety parameters by the official testing laboratories (OMCL), testing of the manufacturing documentation (Lot Release Protocol, LRP) is also part of the scope of the official batch release. The OMCL checks the results of the experimental batch tests carried out by the manufacturer with regard to whether all

critical parameters specified in the marketing authorisation and their thresholds (specifications) have been complied with. The analytical methods used by manufacturers to determine residual amounts of DNA in COVID-19 mRNA active substances are described in the authorisation dossiers of the authorised mRNA vaccine products. Their validity is checked in accordance with guidelines from ICH (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) and proven on the basis of the data provided. Each batch of the vaccine product Comirnaty is tested for residual DNA and the results are part of the manufacturer's batch release protocol, which is independently assessed by the authorities as part of the official batch testing process (OCABR). When it comes to federal batch release in Germany, the test data collected by the manufacturer using a defined and validated method are cross-checked by the Paul-Ehrlich-Institut before the Institute carries out a federal batch release for Germany.

Residual plasmid DNA quantities are deliberately tested on the active substance of the COVID-19 mRNA vaccines (drug substance) and not on the final product (drug product). This is the only way to rule out possible test interference by lipid nanoparticles (LNPs), which are only present in the final product. In the production steps between the production of the active ingredient and the production of the final product, no more DNA can enter the process or the product. This means that no increase in the DNA content per vaccine dose is possible during the production of the final vaccine doses from the active ingredient. Testing the residual DNA on the active substance is therefore more sensitive and representative of the DNA content of the final vaccine product.