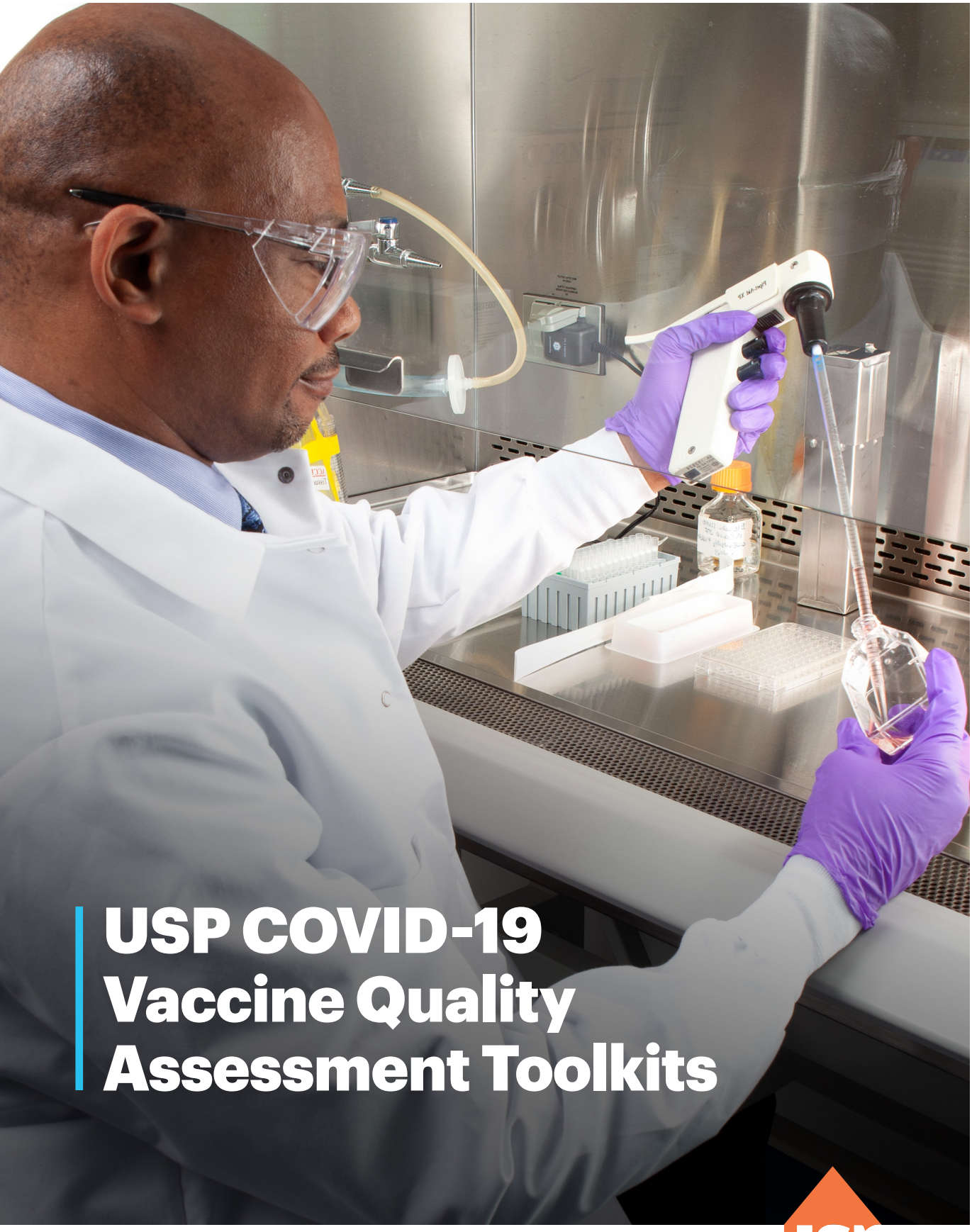
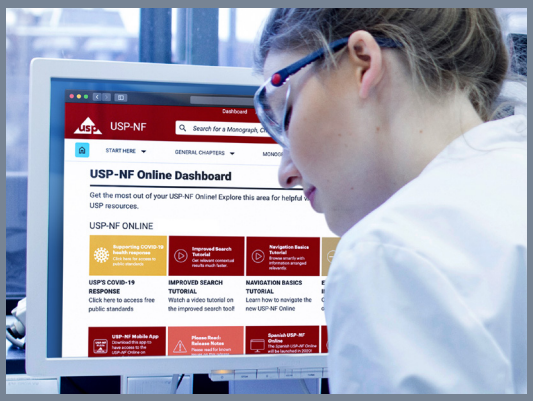


USP COVID-19 Vaccine Quality Assessment Toolkits

January 2022

Introduction

As COVID-19 vaccines are rolled-out globally, it is important to ensure the quality of these vaccines. Validated release assays are important tools to facilitate global distribution of quality vaccines. USP has existing resources, in the form of documentary standards, that can add value to the development and validation of analytical assays to assess various quality attributes of vaccines. We are offering complimentary access to select chapters within the *USP National Formulary (USP-NF)* that can support development and validation of analytical assays.



To register for your complimentary access to the *USP–NF* please follow these instructions:

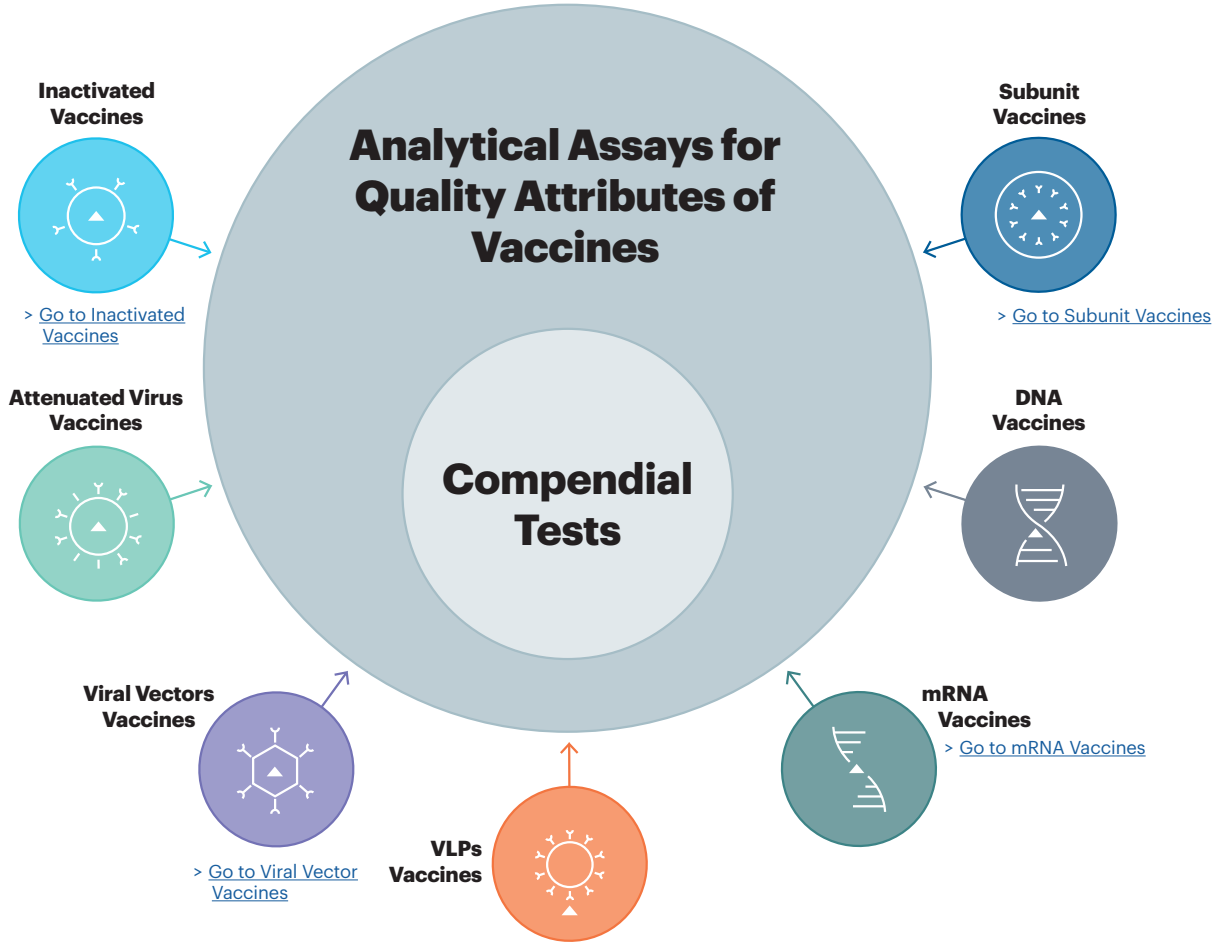
- Go to *USP-NF*. This will take you to the **"USP Welcome to Access Point!"** webpage.
- Click on **"Create a new Access Point account, if you do not have one."** link, near the bottom of the page.
- Complete the information on the **"Create Account"** webpage. Your email address will be your log-in name and you will be able to create a unique password. Please be sure to complete all required fields and click **"Submit".**
- Following submission of your information, you will receive an email to the account you registered to activate your account.
- Click the **"Activation Link"** and your account will be created.
- You will now be able to log into **Access Point** using the email address and password you provided.
- Your initial log-in will take you to the **"Free Resources"** tab of the **"Manage Subscriptions"** page of the ***USP–NF***.
- You can access the complimentary chapters that support the toolkits by clicking on the link for **"Vaccine Quality Assessment Toolkits"** which will take you to the ***USP–NF*** and allow you to view the relevant chapters.
- Once you have registered for your complimentary access to the ***USP–NF***, clicking on the various chapter numbers in the toolkits will now take you directly to that chapter, after you log-in with you complimentary **Access Point** account.

Disclaimer: The resources provided in these toolkits are an informational resource and meant to be supportive for laboratories that need to develop and validate assays for vaccine release. These toolkits are not meant to imply that all the assays are required for release of vaccine and many of these documentary standards resources provide information and best practices for various analytical methods without providing step by step procedures. Follow local laws and cGMPs to determine which assays are required for release of vaccine products. Additionally, parties relying on the information in this document bear independent responsibility for awareness of, and compliance with, any applicable federal, state, or local laws and requirements. These analytical toolkits focus on final vaccine products and not bulk vaccine substances. The key points for these toolkits are:

- The various attributes, methods and resources provided in the toolkits are meant to be for informational purposes and provided as supportive. They do not imply that assessment of all these attributes or use of all these specific methods are required.
- The toolkits are intended for assessment of final vaccine products, and while some attributes and methods may be relevant to bulk vaccine substance, there are assessments for bulk vaccine that are not included in these toolkits.
- Compendial assays relevant to all vaccine platforms (including assays for sterility and endotoxin) are included as a separate Compendial Assay toolkit.
- The toolkits are presented by vaccine platform (e.g., mRNA, viral vector, etc.) and are not meant to be specific to any certain vaccine.
- The toolkits do not include assessment of excipients which are important components of vaccines, but beyond the scope of the toolkits.

Platforms being used to develop vaccines against COVID-19

Toolkits for additional platforms will be added as these vaccines are authorized for COVID-19



Individual Toolkit Contents:

Standards Generally Related to Viral Vaccines and Vaccine Platforms

Title	USP chapters
Injectable Drug Products	<1>
Vaccines for Human Use	<1235>
Viral Vaccines	<1239>
Gene Therapy Products	<1047> [1]
Antimicrobial Effectiveness [2]	<51>
Pharmaceutical Dosage Forms	<1151> [3]
Immunologic Methods	<1102>
Light scattering for particle analysis	<1430>
Excipients [4]	n/a [5]
General Notices and Requirements [6]	n/a

Compendial Tests
that may be applicable to most Vaccine Platforms

Attribute	USP chapters
Appearance	<1>, <790>
pH	<791>
Osmolality and Osmolarity	<785>
Container Closure Integrity	<1207>
Container Content for Injections *(includes extractable volume)*	<697>
Sterility	<71>
Bacterial Endotoxins	<85>

1. Contains information on viral vectors that may be useful for certain vaccines
2. May be included in some multidose vial vaccine presentations
3. Also includes general information on fill volumes and product quality tests
4. Excipients are specific to each vaccine, and while important components of a vaccine, their analysis is beyond the scope of this toolkit. Please review the available literature or contact the vaccine manufacturer for more information about excipients in specific vaccines.
5. Not applicable to specific USP Chapters
6. The General Notices and Requirements section (the General Notices) presents the basic assumptions, definitions, and default conditions for the interpretation and application of the *United States Pharmacopeia (USP) and the National Formulary (NF)*.

Toolkit for Assessing Quality Attributes: mRNA Vaccines

Category	Attribute	Possible Methods [1]	Resource
Identity	Sequence Confirmation	Sequencing	<1125>, <1126>
		RT-qPCR	<1126>, <1127>
Purity	RNA Integrity	CGE	<1053>
		Agarose Gel Electrophoresis for nucleic acids	<1126>
	Product-related impurities	IP-RP-HPLC	<621>
Potency [2]	Antigen expression	Western blot	<1104>
		Flow cytometry	<1027>
		Other cell-based assays	<1032>, <1033>, <1034>
Concentration	RNA Content	RT-qPCR	<1127>
		Fluorescence spectroscopy	<853>
		UV Absorbance	<857>
		Anion exchange chromatography	<1065>
Particle Size	Nanoparticles	Light Scattering	<1430.2>, <1430.3>, <1430.5>, <1430.6>

See Compendial Tests Table on page 3 for additional relevant methods including safety

1. For some methods, the RNA content will need to be extracted from the excipients e.g., lipid capsule
2. The potency of RNA vaccines can be bridged to RNA content during development. Further dialog between manufacturers and regulators may be needed.

Abbreviations: RT, reverse transcriptase; q, quantitative; PCR, polymerase chain reaction; CGE, capillary gel electrophoresis; IP, ion pair; RP, reverse phase; HPLC, high-performance liquid chromatography

Toolkit for Assessing Quality Attributes: Inactivated Vaccines

Category	Attribute	Possible Methods	Resource
Identity and Potency	Detection of immunogenic viral protein epitopes in product	ELISA	<1103>
	Immunogenicity [1]	Immunization of test animals followed by [2] • ELISA • Surface plasmon resonance • Single radial immuno-diffusion to detect antibodies • Virus neutralization[3]	<1032>, <1033>, <1034> <1103> <1105> <90> <1237>
Purity	Residual active virus contamination [4]	Plaque assay	<111>, <1235>, <1237>
	Residual inactivating agent (e.g., formaldehyde or β-propiolactone)		VICH Topic GL25: Testing of Residual Formaldehyde [5] Lei et al. 2018. J. Pharm. Anal. B-Propiolactone Determination
Concentration	Detection of specific/target antigen content (e.g., spike protein)	ELISA	<1103>
	Total protein content	Total protein assay	<1057>, <507>

See Compendial Tests Table on page 3 for additional relevant methods including safety

1. The use of animal-based tests may be required by local statute.
2. In vitro methodology is preferred. The WHO does not recommend the use of in vivo test methods.
3. Requires the use of live pathogenic virus (or a relevant pseudo-virus). Appropriate biosafety precautions must be observed. Only relevant to viral vaccines.
4. Validation of inactivation of the virus is part of development of the manufacturing process and therefore not usually performed on vaccine product
5. These two links are to external resources that are not in the USP-NF. These resources are not published by USP and are provided for informational purposes only.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; GC, gas chromatography; MS, mass spectrometry; RT, reverse transcriptase; PCR, polymerase chain reaction

Toolkit for Assessing Quality Attributes: Viral Vector Vaccines

Category	Attribute	Possible Methods	Resource
Identity	Sequence Confirmation	DNA extraction & sequencing	<1125>, <1126>
		Restriction analysis	<1129>, <1126>
		qPCR, ddPCR, RT-PCR	<1125>, <1126>, <1127>
	Vector detection	ELISA	<1103>
		Various HPLC methods	<621>
Purity	Vector aggregates	Light Scattering	<1430.2>, <1430.3>, <1430.5>, <1430.6>, <1430.7>
		SEC-MALS	<621>, <1430.1>
Potency	Infectious vector titer	Plaque assays	<111>, <1235>, <1237>
		Cell-based assays and tissue culture infectious dose 50%	<1032>, <1033>, <1034>
		Cell-based qPCR	<1032>, <1033>, <1034>
	Detection of transgene expression in cells	Western blot	<1104>
		ELISA	<1103>
		LC-MS	<621>, <736>, <1736>
		RP-HPLC	<621>
Concentration and particle size	Vector particles	Light scattering & DLS	<1430.2>, <1430.6>
		CZE	<1053>
	Nucleic acid content [1]	qPCR	<1125>, <1126>, <1127>

See Compendial Tests Table on page 3 for additional relevant methods including safety

1. May also be applicable to Potency.

Abbreviations: q, quantitative; PCR, polymerase chain reaction; dd, droplet digital; RT, real time; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; SEC, size exclusion chromatography; MALS, multi-angle light scattering; LC, liquid chromatography; MS, mass spectroscopy; DLS, dynamic light scattering; CZE, capillary zone electrophoresis;

Toolkit for Assessing Quality Attributes: Protein Subunit Vaccines

Category	Attribute	Possible Methods [1]	Resource
Identity	Detection of antigenic subunit(s) in product [2]	ELISA	<1103>
		Western Blot	<1104>
		HPLC with UV or MS detection	<621>, <736>, <1736>
		Peptide mapping	<1055>
Purity	Protein Purity [6]	SDS-PAGE	<1056>
		HPLC [7]	<621>
		cIEF	<1054>, <1053>
Potency	Immunogenicity [3]	ELISA for specific antigen epitopes	<1103>
		Immunization of test animals followed by: [4] • ELISA • Surface plasmon resonance • Virus neutralization [5]	<1032>, <1033>, <1034> <1103> <1105> <1237>
Concentration	Protein content	Total Protein assays	<1057>, <507>
	Quantitation of antigenic content	ELISA	<1103>
	Percent adsorption of vaccine antigen to adjuvant [8]	Free protein vs. Total reported protein [9]	<1057>, <507>

See Compendial Tests Table on page 3 for additional relevant methods including safety

1. Adjuvants in some vaccine formulations may interfere with some methods and extraction of the vaccine antigen(s) may be necessary prior to testing.
2. Some methods may not have the resolution to differentiate between vaccines against variants of the primary antigen. Peptide mapping would be recommended for such an application.
3. In vitro methodology is preferred. The WHO does not recommend the use of in vivo test methods.
4. The use of animal-based tests may be required by local statute. The serum from the immunized animals would be the test samples for this application.
5. Requires the use of live pathogenic virus (or a relevant pseudo-virus). Appropriate biosafety precautions must be observed. Only relevant to viral vaccines.
6. Purity of the primary protein antigen in terms of possible aggregates or degradation products as well as other potentially contaminating proteins.
7. Various HPLC methods may be applicable for detection of potential impurities.
8. This is relevant to certain adjuvants like aluminum.
9. May be performed using a modification of the Lowry method following separation of the absorbed and unabsorbed vaccine antigen.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; UV, ultraviolet; MS, mass spectroscopy; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; cIEF, capillary isoelectric focusing