Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting Viral Pathogens
Guidance for Industry

DRAFT GUIDANCE

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For questions regarding this draft document, contact Natalia Comella at 301-796-6226.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

March 2023
Pharmaceutical Quality/CMC

Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting Viral Pathogens

Guidance for Industry

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Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting Viral Pathogens Guidance for Industry[1]

> This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

The purpose of this guidance is to provide to sponsors recommendations that assist in the development of monoclonal antibodies (mAbs) and other therapeutic proteins[2] that directly target viral proteins or host cell proteins mediating pathogenic mechanisms of infection.[3] A critical quality control measure for these products is the development and implementation of a potency assay(s)[4] adequate to ensure that each lot is produced consistently with the potency[5] necessary to achieve clinical efficacy and that such potency is maintained over the shelf life of the product. This guidance provides detailed recommendations to drug developers with the goal

[1] This guidance has been prepared by the Office of Pharmaceutical Quality in the Center for Drug Evaluation and Research at the Food and Drug Administration.

[2] The term *protein* is one of the statutory categories of biological products (section 351(i)(1) of the Public Health Service Act (42 U.S.C. 262(i)(1))). Under 21 CFR 600.3(h)(6), a protein is any alpha amino acid polymer with a specific, defined sequence that is greater than 40 amino acids in size.

[3] In January 2021, FDA published the guidance for industry *COVID-19: Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting SARS-CoV-2 Infectivity*, which focuses solely on addressing potency assays as they relate to mAbs and other therapeutic proteins that directly target SARS-CoV-2. That guidance states it is intended to remain in effect only for the duration of the public health emergency related to Coronavirus Disease 2019 declared by the Secretary of Health and Human Services under section 319 of the Public Health Service Act (section 319 public health emergency). FDA is issuing this draft guidance because many of the recommendations set forth in the 2021 guidance are applicable outside the context of the section 319 public health emergency and are applicable to mAbs and other therapeutic proteins directly targeting any viral surface (glyco)proteins mediating pathogenic mechanisms of infection, not just SARS-CoV-2.

[4] See 21 CFR 610.10.

[5] Under 21 CFR 600.3(s), *potency* is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.

26 of helping to ensure that drug developers provide adequate information to assess potency at each
27 stage of a product's life cycle.
28
29 This guidance applies only to mAbs and other therapeutic proteins regulated by the Center for
30 Drug Evaluation and Research that are designed to bind to viral proteins or their receptors on
31 host cells, inhibit viral entry, and/or elicit Fc-mediated effector function, and are subject to
32 licensure under section 351(a) or section 351(k) of the Public Health Service Act (42 U.S.C.
33 262(a) or (k)). This guidance does not apply to other biological products such as
34 immunomodulatory drugs (e.g., cytokines or cytokine antagonists), vaccines, hyperimmune
35 globulins, gene therapies, cell therapies, and convalescent plasma.[6]
36
37 The guidance describes approaches that sponsors should use to develop potency assay methods
38 for release and stability that assess comprehensively known or potential mechanism(s) of action
39 of the product. The sensitivity of such methods must be established,[7] for example, to conduct
40 the appropriate laboratory determination of satisfactory conformance to final specifications for
41 the drug product (i.e., to demonstrate lot-to-lot consistency). In addition to release and stability
42 methods, other methods that demonstrate the biological function(s) of the product may be needed
43 for characterization and comparability studies. The guidance describes methods that sponsors
44 should use to ensure the potency of mAbs and other therapeutic proteins intended to prevent or
45 treat a viral infection.
46
47 Although assays and model systems vary with different viruses, the principles in this guidance,
48 where applicable, are relevant to mAbs and other therapeutic proteins under development for the
49 prevention or treatment of viral infections.[8] Because this field is dynamic and continually
50 evolving, the principles are also intended to be flexible to accommodate new assays or
51 technologies. If circumstances warrant, this guidance may be revised to inform sponsors of
52 additional methodologies to consider.
53
54 In general, FDA's guidance documents do not establish legally enforceable responsibilities.
55 Instead, guidances describe the Agency's current thinking on a topic and should be viewed only
56 as recommendations, unless specific regulatory or statutory requirements are cited. The use of
57 the word *should* in Agency guidances means that something is suggested or recommended, but
58 not required.
59
60

[6] Manufacturers of vaccines and certain medical devices (e.g., in vitro diagnostics) should consult the center review offices regarding appropriate assays and methods for products regulated by the Center for Biologics Evaluation and Research and the Center for Devices and Radiological Health.

[7] See 21 CFR 211.165(a) and (e), 21 CFR 601.2(d), and 21 CFR 610.10.

[8] As an example, some mAbs do not demonstrate both virus neutralization and Fc-mediated effector functions as their mechanisms of action; therefore, only the principles relevant to the particular mAb would apply under those circumstances.

61 **II. BACKGROUND**
62
63 The Agency receives investigational new drug applications (INDs) and biologics license
64 applications (BLAs) for mAbs and other therapeutic proteins designed to bind to viral surface
65 (glyco)proteins or their receptors on host cells, inhibit viral entry, and/or elicit Fc-mediated
66 effector function. Monoclonal antibodies and mAb cocktails[9] being developed to target viral
67 pathogens, including newly emerging viruses, generally use mechanisms of action that may
68 include virus neutralization, Fc-mediated effector functions, or both. Monoclonal antibodies
69 may neutralize the virus by binding the viral attachment protein or the host cell receptor (e.g.,
70 angiotensin-converting enzyme 2 for coronaviruses, sialic acid for influenza), thereby blocking
71 attachment to host cell receptors; may prevent the fusion of the viral and host cell membranes
72 (e.g., respiratory syncytial virus F protein); and/or may inhibit other viral or host factors
73 necessary for entry. These products are referred to as neutralizing mAbs. Some mAbs directed
74 against viruses mediate Fc-effector functions in addition to or instead of neutralizing virus entry.
75 Other mAbs may target alternative cellular receptors or cellular proteins that facilitate virus
76 infection.
77
78 In addition to mAbs, other protein therapeutics intended to target viral entry may be developed to
79 prevent or treat viral infection. These include scaffold proteins, which are engineered to have
80 similar mechanisms as neutralizing mAbs, bifunctional molecules engineered to interfere with
81 different steps of viral entry, or recombinant virus receptor proteins, which serve as decoy
82 receptors to inhibit viral infectivity. As the science evolves, other novel proteins with other
83 mechanism(s) of action may also be developed and submitted to the Agency.
84
85
86 **III. POTENCY ASSAY CONSIDERATIONS**
87
88 To ensure mAbs and therapeutic proteins' clinical effectiveness through product expiry, a
89 sponsor must develop a method or methods to monitor the potency of the biological product.[10]
90 Potency assays for mAbs and therapeutic proteins should be designed to measure the binding to
91 viral receptors on host cells, the inhibition of viral entry, and/or to elicit Fc-mediated effector
92 function. Potency assays also should be designed to reflect the biological activity of the mAb or
93 therapeutic protein in vivo. Potency measurements from these methods should be used to
94 demonstrate that only product lots that meet defined specifications or acceptance criteria are
95 administered during all phases of clinical investigation and after approval.
96

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[9] In general, the term *mAb cocktails* refers to two or more mAbs administered at a fixed ratio. They may be filled in a single vial or separate vials. For the purposes of this guidance, the term refers to two or more mAbs filled in a single vial.

[10] See 21 CFR 610.10.

97 All potency assays used for release and stability testing must comply with applicable biologics
98 and current good manufacturing practice regulations.[11,12] When evaluating the appropriateness
99 of a potency assay for a specific mAb or other therapeutic proteins for treating or preventing a
100 viral infection, FDA may consider various factors, including: (1) product characteristics; (2)
101 manufacturing processes; (3) the stage of development in which the assay will be used; (4) the
102 strength of the sponsor's risk-based quality assessment; and (5) the totality of the information
103 provided by the sponsor.
104
105
106 **IV. MEASURING AND MONITORING POTENCY CONSIDERATIONS**
107
108 Sponsors developing mAbs or other therapeutic proteins for the treatment and/or prevention of a
109 viral infection should implement potency assays for release and stability testing specifically
110 designed to demonstrate the biological function(s) of the product[13] and provide justification for
111 how assays used for release and stability testing measure comprehensively known or potential
112 mechanism(s) of action of the product. Sponsors should develop a manufacturing control
113 strategy that will identify potential shifts in the product's critical quality attributes known to
114 affect each known and/or potential mechanism of action and detect changes in the performance
115 of the manufacturing process before beginning phase 3 trials.
116
117 Depending on the proposed mechanism(s) of action, one or more assays should be developed to
118 support the control strategy for confirming the biologic's potency.[14] If the mAb or other
119 therapeutic protein has multiple mechanisms of action (e.g., neutralization and Fc-effector
120 function), multiple potency assays should be used. All quality-control release test methods,
121 including potency assays for demonstrating the mechanism(s) of action, should be shown to be
122 suitable for their intended purposes during development and must be validated by the time of a

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[11] See, for example, 21 CFR 610.10 (regarding tests for potency for biological products); 21 CFR 211.165(e) (regarding the establishment and documentation of the accuracy, sensitivity, specificity, and reproducibility of test methods); 21 CFR 211.160(b) (regarding laboratory controls); and 21 CFR 211.194(a)(2) (regarding laboratory records of testing methods).

[12] Sponsors should consider potency assay development in their overall drug development program. The IND regulations at 21 CFR 312.23(a)(7)(i) require that, in each phase of the investigation, sponsors submit sufficient information "to assure the proper identification, quality, purity, and strength of the investigational drug," and indicate that "the amount of information needed to make that assurance will vary with the phase of the investigation, the proposed duration of the investigation, the dosage form, and the amount of information otherwise available." For additional information, see the guidances for industry *Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products* (November 1995) and *INDs for Phase 2 and Phase 3 Studies; Chemistry, Manufacturing, and Controls Information* (May 2003). We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[13] See the ICH guidance for industry *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* (August 1999).

[14] Ibid.

123 BLA submission.[15] The development of quality-control release methods should be informed by
124 the product characterization. Special consideration should be given for emerging or highly
125 pathogenic viruses. Additional parameters may need to be incorporated into the development of
126 the potency assay(s). Sponsors should consult FDA for further guidance.[16]
127
128 This section provides examples of assays, depending on the mechanism(s) of action, that can be
129 included as part of the overall control strategy.
130
131 **A. Methods**
132
133 Because a binding assay demonstrates binding between the mAb or therapeutic protein and its
134 target, it is generally sufficient to serve as a potency assay at the early stages of drug
135 development. However, a binding assay assesses only one aspect of the potency of a product.
136 Therefore, sponsors should subsequently develop methods that more comprehensively monitor
137 the proposed mechanism(s) of action of the products. These methods should be incorporated
138 into drug substance and drug product release testing and stability protocols. Potency assays
139 should be described, justified, qualified, and validated to support a BLA.
140
141 This section addresses considerations for possible assays.
142
143 *1. Binding Assays*
144
145 For the purposes of this guidance, *binding assays* are defined as assays that quantify the binding
146 between the mAb or other therapeutic protein and its target. These assays are established early
147 in product development, typically in the form of a direct binding assay such as an enzyme-linked
148 immunosorbent assay (ELISA) or a surface plasmon resonance (SPR) assay. Product lots should
149 be compared to an appropriately qualified in-house reference material and activity should be
150 expressed as a percentage of the reference material value. Although helpful in the initial phases
151 of development, these assays do not directly confirm the product's ability to inhibit the target
152 protein's activity and should not be used in lieu of methods that confirm potency.
153
154 For products intended to inhibit viral protein binding to a host cell receptor, FDA recommends a
155 potency assay that is a better reflection of the intended mechanism of action instead of a direct
156 binding assay; for example, an inhibition assay, such as an inhibition ELISA,[17] or SPR. An

[15] See 21 CFR 211.165(e) and as described in the guidance for industry *Analytical Procedures and Methods Validation for Drugs and Biologics* (July 2015). See also the ICH draft guidance for industry *Q2(R2) Validation of Analytical Procedures* (August 2022); when final, this guidance will represent the FDA's current thinking on this topic.

[16] Although this guidance covers in vitro approaches for potency assays, there may be situations in which an in vivo assay is the only approach that can ensure product quality; however, these situations are rare and should be discussed with the Agency.

[17] See, for example, Tan, CW, WN Chia, X Qin, P Liu, MI-C Chen, C Tiu, Z Hu, VC-W Chen, BE Young, WR Sia, Y-J Tan, R Foo, Y Yi, DC Lye, and DE Anderson, 2020, A SARS-CoV-2 Surrogate Virus Neutralization Test Based on Antibody-Mediated Blockage of ACE2–Spike Protein–Protein Interaction, Nat Biotechnol.

157 inhibition assay should be designed to evaluate the inhibition of virus-receptor interactions and
158 may be appropriate to conduct in, for example, either a biosafety level (BSL)-1 or BSL-2 lab.[18]
159
160 *2. Viral Neutralization Assays*
161
162 In comparison to binding assays, in vitro viral neutralization assays more comprehensively
163 confirm a mAb's or therapeutic protein's mechanism of action and potency in blocking infection
164 of susceptible cells. Because of the potential importance to evaluating these products, the
165 Agency recommends establishing an in vitro viral neutralization assay early in development.
166 This type of assay can be useful for advancing development, quality control, and characterization
167 of neutralizing mAbs and other products targeting viral attachment and entry. Given the
168 diversity of mechanisms for viral attachment and entry into host cells, the assay should reflect
169 that virus's mechanisms for attachment and entry.
170
171 Assays that assess the ability of the mAbs or other therapeutic proteins to inhibit any of the
172 binding or entry steps are predominantly cell-based assays and typically involve the use of wild-
173 type (wt) virus,[19] pseudotyped virus, or pseudotyped virus-like particles (VLP). When
174 considering which method to use, sponsors should select a method that best monitors the
175 binding/entry step the product is expected to target in the virus replication cycle. Although wt
176 virus neutralization assays are considered the gold standard for in vitro potency assays,
177 alternative methods may be acceptable. For example, a potency assay could be designed to
178 characterize the effect of the product on a specific entry step (e.g., virus-cell fusion).
179 Additionally, accessibility to appropriate BSL laboratories, as well as challenges to qualifying
180 critical reagents and validating the overall assay performance, should be considered in assay
181 selection. For methods using transfected cell lines, sponsors should also address target cell
182 viability and variability. Whichever method is ultimately used, sponsors should observe all
183 provisions of the select agent regulations[20] (if applicable) and other applicable governmental and
184 institutional biosafety and biosecurity provisions.
185

[18] Relevant biosafety considerations may be found in the National Institutes of Health's guidelines *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (April 2019) (available at https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf) and on the FAQ web page Interim Laboratory Biosafety Guidance for Research with SARS-CoV-2 and IBC Requirements under the NIH Guidelines (https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy/interim-laboratory-biosafety-guidance-for-research-with-sars-cov-2-and-ibc-requirements-under-the-nih-guidelines/) and the Centers for Disease Control and Prevention's guideline *Biosafety in Microbiological and Biomedical Laboratories* (available at https://www.cdc.gov/labs/BMBL.html).

[19] For the purposes of potency assays discussed in this guidance, references to wt virus indicate a clinical isolate, which has not been intentionally modified, except for mutations that may occur naturally during in vitro virus passaging and expansion, compared to the initial isolate. Use of lab-adapted viruses should be appropriately justified.

[20] See 7 CFR part 331, 9 CFR part 121, and 42 CFR part 73.

186 Below is a list of assays that may be suitable for use as a potency assay, along with key
187 considerations for each assay:
188
- 189 **Virus neutralization assays.** Depending on the virus, these assays (such as plaque
190 reduction, $TCID_{50}$, and microneutralization assay) involve working in the appropriate
191 BSL laboratory. If using minimally passaged wt virus or a lab-adapted isolate(s),
192 sponsors should determine virus titer and develop, aliquot, and store master and working
193 virus stocks appropriately. If using a lab-adapted isolate(s), a sponsor should indicate the
194 isolate used and justify the use of that isolate(s) in applicable submissions to FDA.
195 Sponsors should provide details on the production, storage condition, and stability of
196 virus stocks used in the assays as discussed in existing guidance on these topics.[21] The
197 qualification of any new working stock should include the sequencing of the epitope.
198
- 199 **Pseudotyped virus- or VLP-based assay.** An alternative to working with a wt virus is
200 the implementation of pseudotyped virus-based methods. These methods should be
201 performed under appropriate biosafety conditions.[22] Pseudotyped virions can be
202 generated by replacing the surface protein(s) expressing gene of a less pathogenic virus
203 (e.g., vesicular stomatitis virus) with the gene encoding the viral surface
204 (glyco)protein(s)[23] of the pathogen of interest, creating engineered, replication-competent
205 virions. Another approach is to generate fusion-competent, but replication-incompetent
206 VLPs by co-transfecting producer cells (usually 293T cells) with a set of plasmids
207 encoding the viral surface (glyco)protein(s) and a matrix protein(s) driving the VLP
208 budding in trans. Retrovirus-based VLP packaging systems have already been adapted
209 for viral surface (glyco)protein(s) pseudotyping.
210
211 During method development of a neutralization assay using a pseudovirus or VLP,
212 sponsors should generate data demonstrating viral surface (glyco)protein-mediated cell
213 entry and describe the generation, isolation, purification, and concentration steps used to
214 minimize lot-to-lot variability of the pseudovirus or VLP. Additionally, sponsors should
215 address the following:
216
  - 217 The critical reagents (i.e., producer and target cell lines, and virion constructs, as well
218 as controls (negative and positive)) used in the assay should be described. When

[21] See the References section for a list of guidances regarding analytical procedures, method validation, and documentation.

[22] Relevant biosafety considerations may be found in the National Institutes of Health's guidelines *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (April 2019) (available at https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf) and on the FAQ web page Interim Laboratory Biosafety Guidance for Research with SARS-CoV-2 and IBC Requirements under the NIH Guidelines (https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy/interim-laboratory-biosafety-guidance-for-research-with-sars-cov-2-and-ibc-requirements-under-the-nih-guidelines/) and the Centers for Disease Control and Prevention's guideline *Biosafety in Microbiological and Biomedical Laboratories* (available at https://www.cdc.gov/labs/BMBL.html).

[23] In the context of this guidance, the term *viral surface (glyco)protein(s)* refers to virus components (molecules) involved in the virus binding and/or entry into susceptible cells.

qualifying these critical reagents, sponsors should demonstrate long-term stability and address possible variability associated with the transfected cells lines.

  - Attention should be given to the manufacturing, stability, and qualification of the pseudovirus or VLP, which is a critical reagent for the assay. Lot-to-lot variability of VLPs can be observed in the quantity of the VLPs and the activity of the VLP stock. Sponsors should demonstrate VLP lot-to-lot consistency is appropriately controlled.

- **Viral surface (glyco)protein(s)-mediated cell-cell fusion-based assays**. These assays can be used as an alternative to the pseudotyped virus- or VLP-based assays. Typically, the level of fusion between the viral surface (glyco)protein(s)-expressing cells and the virus receptor-expressing cells should be assessed using a reporter gene. The expression of the reporter construct is dependent on the successful fusion between the two cell populations. Alternatively, the level of syncytia formation or dye transfer also can be used to quantify cell-cell fusion. As with the pseudotyped virus-based assays, the following should be addressed:

  - The controls (negative and positive), cell lines, constructs, and reporter constructs used in the method should be described. When qualifying these critical reagents, sponsors should demonstrate their long-term stability and address possible variability associated with the transfected cells lines.

  - How well the assay mimics viral-cell fusion should be described. For example, if certain conformational or environmental (e.g., pH) changes are needed for fusion to occur, the assay should monitor those changes.

  - The testing platform to be used with the assay should be indicated.

  - Defined quantitation criteria should be used regardless of whether a reporter construct, syncytia formation, or dye transfer is used to measure cell-cell fusion.

Given possible differences between wt virus neutralization assay(s) and the alternative methods mentioned above (pseudotyped virus neutralization methods, VLP-based methods, or viral surface (glyco)protein(s)-mediated cell-cell fusion-based assays), FDA recommends that sponsors provide information addressing how (or whether) the assay's results correlate with wt virus neutralization.

Although this section of the guidance focuses on neutralizing assays for products that block viral surface (glyco)protein(s)-mediated virus entry, the concepts described herein are applicable to the development of mAbs or other therapeutic proteins that block other steps in the virus replication cycle.

261 *3. Fc-effector Function Assays*
262
263 In general, sponsors should assess mAbs for their ability to demonstrate Fc-mediated effector
264 functions.[24] This can include complement activity and activities mediated through binding to
265 Fcγ receptors, such as antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-
266 dependent cellular phagocytosis. For mAbs demonstrating Fc-effector functions, appropriate
267 methods should be included as part of the specifications to ensure consistent mAb potency and
268 functions. Fc glycosylation relevant to the mechanism of action should also be monitored and
269 controlled throughout the product life cycle. FDA recommends including an FcγRIIIa-
270 mediated/natural killer cell ADCC assay because that appears to be the most sensitive to changes
271 in glycosylation.
272
273 For mAbs engineered to alter binding to Fc receptors and complement components,
274 characterization studies should be conducted on a one-time basis to demonstrate the engineered
275 mAb performs as designed. Fc-fusion proteins may also engage Fc receptors and complement
276 components and thus should also be characterized for these potential effects or to confirm the
277 intended effect of any Fc modifications. For example, the Agency recommends that virus
278 receptor-Fc fusion proteins be characterized for their ability to carry out Fc-effector functions
279 using methods similar to those for the characterization of neutralizing mAbs.
280
281 **B. Additional Considerations**
282
283 Sponsors should address the following additional considerations when developing methods to
284 monitor potency.
285
286 • When describing a potency assay, sponsors should ensure that the virus isolate, or viral
287 surface (glyco)protein(s), used reflects common isolates prevalent in the United States.
288 Sponsors should discuss how the isolates or proteins were selected and whether they
289 reflect the viruses currently in circulation. Sponsors also should provide either the full
290 genome sequence(s) of the isolate(s) or GenBank ID(s).
291
292 • Whether using wt virus, pseudotyped virus, or VLPs, a master cell bank of producer cells
293 should be appropriately qualified and used to generate a working bank of virus producer
294 cells.
295
296 • Information and data submitted to support BLAs should describe any differences in the
297 methods used for release testing and stability program compared to those used to initially
298 characterize the potency of the mAb or other therapeutic protein during earlier
299 development. That includes, but is not limited to, reagents, testing site(s), and testing
300 platform(s), if applicable, to conduct the assay in question.
301

[24] For more information on Fc-mediated effector functions, see, for example, Jiang, X, A Song, S Bergelson, T Arroll, B Parekh, K May, S Chung, R Strouse, A Mire-Sluis, and M Schenerman, 2011, Advances in the Assessment and Control of the Effector Functions of Therapeutic Antibodies, Nature Reviews; Drug Discovery 10:101-110.

302 - Relevant positive and negative controls should be included as part of the potency assay.
303
304 - For mAb cocktails, release testing methods should include an identity method that
305 demonstrates the presence of each individual mAb and a quantitative method verifying
306 the ratio of the individual mAbs. The sponsor should ensure the ratio is consistent from
307 lot to lot.
308
309 Novel mechanisms of action that are not addressed in this guidance may be identified in the
310 future. For mAbs or other therapeutic proteins with such novel mechanisms of action, sponsors
311 should consult the Agency for further recommendations regarding compliance with requirements
312 for potency assays for release and stability testing.[25]

[25] See 21 CFR 610.10, 211.165(e), and 211.194(a)(2).

REFERENCES

Analytical Procedures, Method Validation, and Documentation-Related Guidances for Industry[26]

- Guidance for industry *Analytical Procedures and Methods Validation for Drugs and Biologics* (July 2015)
- Guidance for industry *Bioanalytical Method Validation* (May 2018)
- Guidance for industry *Content and Format of Investigational New Drug Applications (INDs) for Phase 1 Studies of Drugs, Including Well-Characterized, Therapeutic, Biotechnology-Derived Products* (November 1995)
- Guidance for industry *INDs for Phase 2 and Phase 3 Studies; Chemistry, Manufacturing, and Controls Information* (May 2003)
- Guidance for industry *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* (February 1997)
- ICH draft guidance for industry *Q2(R2) Validation of Analytical Procedures* (August 2022)[27]
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- ICH guidance for industry *Q5C Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products* (July 1996)
- ICH guidance for industry *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products* (August 1999)

Centers for Disease Control and Prevention Guideline[28]

- *Biosafety in Microbiological and Biomedical Laboratories*

Literature

- Jiang, X, A Song, S Bergelson, T Arroll, B Parekh, K May, S Chung, R Strouse, A Mire-Sluis, and M Schenerman, 2011, Advances in the Assessment and Control of the Effector Functions of Therapeutic Antibodies, Nature Reviews; Drug Discovery 10:101-110.

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[27] When final, this guidance will represent the FDA's current thinking on this topic.

[28] Available at https://www.cdc.gov/labs/BMBL.html.

- Tan, CW, WN Chia, X Qin, P Liu, MI-C Chen, C Tiu, Z Hu, VC-W Chen, BE Young, WR Sia, Y-J Tan, R Foo, Y Yi, DC Lye, and DE Anderson, 2020, A SARS-CoV-2 Surrogate Virus Neutralization Test Based on Antibody-Mediated Blockage of ACE2–Spike Protein–Protein Interaction, Nat Biotechnol.

National Institutes of Health Guidelines

- *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (April 2019)[29]

- FAQ web page Interim Laboratory Biosafety Guidance for Research with SARS-CoV-2 and IBC Requirements under the NIH Guidelines (https://osp.od.nih.gov/policies/biosafety-and-biosecurity-policy/interim-laboratory-biosafety-guidance-for-research-with-sars-cov-2-and-ibc-requirements-under-the-nih-guidelines/)

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[29] Available at https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf.

[30] We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.