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## Title Page Template

- **Article title:** Biomarker assay validation for clinical trials: a questionnaire survey for the pharmaceutical companies in Japan
- **Short running title:** Biomarker assay validation: a survey in Japan
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conflict with, the subject matter or materials discussed in the manuscript other than those disclosed. No writing assistance was utilized in the production of this manuscript.

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1 **Abstract**

2 Not applicable, as this manuscript is commentary.

3

4 **Keywords**

5 Japan, biomarker, clinical trials, assay validation, standard operating procedure

6

7 **Introduction**

8 The pharmaceutical industry has been working on improving drug development since  
9 decades. Currently, lack of efficacy and safety concerns are the major causes of attrition  
10 during late drug development. Under the current situation, biomarkers including  
11 pharmacodynamics (PD) and safety biomarkers play an important role. Hayashi et al.  
12 proved that the use of a biomarker resulted in higher success rates during anticancer drug  
13 development [1]. The discovery and use of biomarkers have been facilitated by recent  
14 advancements in omics technology, establishment of databases, and understanding of  
15 pathophysiology, etc. [2–4]. In the field of quantitative bioanalysis in drug development,  
16 scientists have been working on the delivery of reliable quantitative data on biomarkers  
17 with limited resources in a timely manner. However, there appears to be room for  
18 improvement.

19 To deliver quantitative data of high quality, scientists conduct assay validation. While  
20 some biomarker assays use the same methodologies (e.g. ligand binding assays and  
21 chromatographic assays) as pharmacokinetics (PK) assays, there are some differences in  
22 points to consider in assay validation between biomarker assays and PK assays. Therefore,  
23 biomarker assay validation has been a hot topic in bioanalysis conferences and  
24 communities. American Association of Pharmaceutical Sciences (AAPS) and Clinical  
25 Ligand Assay Society organized a workshop on this subject in 2003, and the meeting  
26 outcome was published in 2006 [5]. European Bioanalysis Forum (EBF) formed its  
27 biomarker team in 2010 [6]. In 2015, Japan Bioanalysis Forum (JBF) Biomarker Task  
28 Force prepared a concept paper on the biomarker analysis during late clinical  
29 development [7], and AAPS and Food and Drug Administration (FDA) held Crystal City  
30 IV meeting [8].

31 However, to our knowledge, there has been only one survey assessing the status of  
32 biomarker assays in industry. The only survey was conducted within European and North  
33 American CROs by Global CRO Council for Bioanalysis (GCC) in 2012 [9]. To  
34 investigate the current situation in the pharmaceutical companies, the biomarker working  
35 group (WG) in the bioanalytical assay validation study group, which was subsidized by  
36 the Japan Agency for Medical Research and Development, decided to conduct a  
37 questionnaire survey in Japan [10]. A part of the survey results was already presented at  
38 the 9<sup>th</sup> JBF symposium in Tokyo, Japan in 2018 and 12<sup>th</sup> Workshop on Recent Issues in  
39 Bioanalysis in Philadelphia, PA, USA in 2018.

40

41 **Survey methods**

42 A survey in an Excel format in Japanese language was prepared by biomarker WG and  
43 distributed to all the member companies of Japan Pharmaceutical Manufacturers  
44 Association (JPMA; 71 companies) in July 2017. Generic pharmaceutical companies and  
45 contract research organizations (CRO) are not members of JPMA. All survey responses  
46 were collected by September 2017 and blinded by the secretariat of biomarker WG before  
47 analysis.

48 The types of survey questions were company-based questions, new medical entity  
49 (NME)-based questions, and assay-based questions. For the company-based questions,  
50 only one response was collected from each company. For the other two questionnaire  
51 groups, each company was encouraged to provide multiple responses.

52

53 **Survey results**

54 Company-based questions

55 Thirty-seven (37) companies responded to the survey. Of these respondent companies, 26  
56 identified themselves as Japanese companies and 11 identified as non-Japanese  
57 companies.

58 First, we asked them about their experiences of biomarker assays in and after 2012.  
59 About a third (32.4%) of all the respondent companies had submitted the quantitative  
60 biomarker data in new drug application (NDA), whereas only a small percentage (5.4%)  
61 of all the respondent companies received questions on the validity of biomarker assays  
62 and sample analysis during the interaction with the regulatory agency. Most (62.2%) of  
63 all the respondent companies had conducted biomarker (except for laboratory tests and  
64 analysis of DNA, RNA, and metal) analysis for clinical trials. This result was similar  
65 between the Japanese and non-Japanese companies. Of those who conducted clinical  
66 biomarker analysis, 34.8% had one NME for the clinical biomarker analysis whereas  
67 other companies had multiple NMEs.

68 Then, we asked about their standard operating procedures (SOPs) on the clinical  
69 biomarker analysis. Some (19.2%) of the Japanese companies and most (63.6%) of the  
70 non-Japanese companies had SOPs, indicating clear differences between the Japanese and  
71 non-Japanese companies. In addition, all SOPs of the Japanese companies did not provide  
72 guidance on assay validation (such as test items and acceptance criteria) whereas majority  
73 of the SOPs of the non-Japanese companies did so. Interestingly, two-thirds of the  
74 Japanese companies without SOPs considered that it is necessary to have SOPs  
75 somewhere in future whereas a third of them did not.

76

77 NME-based questions

78 Fifty-two (52) NMEs were included in the present survey. It is not rare (26.9%) that the  
79 clinical development of one NME was associated with multiple biomarkers.

80

81 Assay-based questions

82 In the present survey, the total number of biomarker assays was 82. Vast majority (82.9%)  
83 of the assays were for PD biomarkers, and only a few (3.7%) were for safety biomarkers.  
84 The other assays (13.4%) included those for patient stratification. For the intended use of  
85 biomarker data, 32.9% of the assays were for claiming characteristics of NME at NDA  
86 submission and the others were for exploration (29.3%) or sponsor decision making  
87 (35.4%).

88 Ligand binding assays and chromatographic assays accounted for 62.2% and 20.7% of  
89 the assays, respectively. The other assays (17.1%) included flow cytometry,  
90 immunohistochemistry, and enzymatic methods. Majority (74.5%) of the ligand binding  
91 assays used commercial kits whereas others (25.5%) were developed de novo. Unlike  
92 ligand binding assays, a vast majority (82.4%) of chromatographic assays were  
93 developed de novo.

94 Reference standards used in ligand binding assays were mostly recombinant proteins  
95 (64.9%), followed by a surrogate analyte such as the signature peptide of protein analytes  
96 (13.5%) and the same chemical as an analyte (5.4%). Vast majority (88.2%) of the  
97 reference standards used in chromatographic assays were the same chemical as an  
98 analyte.

99 Quantitative biomarker analysis is most likely to involve the preparation of samples at  
100 known concentrations, such as calibration samples. As biomarkers are endogenous, the  
101 same biological matrix as study samples often contains a variable concentration of  
102 analyte, and is therefore, not always the best matrix used for this purpose. According to  
103 the present survey, the same biological matrix as study samples was used in 39.0% of the  
104 assays whereas the surrogate matrix (such as water, buffer, solution included in  
105 commercial kits, protein solution, stripped matrix) was used in 37.8% of the assays. For  
106 the other assays (23.2%), the respondents indicated that this question was not applicable  
107 to the assay or did not provide an answer.

108 The survey results on the validation items are shown in Supplementary Figure 1 (per  
109 methodology). For more than half of the ligand binding assays, respondents examined  
110 selectivity, calibration curve, lower limit of quantification (LLOQ), upper limit of  
111 quantification (ULOQ), accuracy, “precision in the same matrix as study samples”,  
112 matrix stability, and dilution linearity/dilution integrity. For more than half of the  
113 chromatographic assays, respondents examined selectivity, calibration curve, LLOQ,  
114 ULOQ, accuracy, “precision in surrogate matrix”, “precision in the same matrix as study  
115 samples”, matrix effect, carryover, matrix stability, and dilution linearity/dilution integrity.  
116 Parallelism has been deemed as an important validation item in some white papers  
117 [8,9,11]. Evaluation of parallelism is considered critical if the surrogate matrix or  
118 surrogate analyte (for example, a recombinant protein and a stable isotope-labelled  
119 analyte) is used. In the present survey, surrogate matrix and recombinant protein were  
120 used in 37.8% and 34.1% of the assays, respectively; however, parallelism was examined

121 only in few cases (16.7% of the ligand binding assays and 0.0% of the chromatographic  
122 assays). A free-text question revealed that recovery, hook effect, processed sample  
123 stability, and cross validation (between different methods or different laboratories) were  
124 examined in some cases.

125 To investigate the determinant of validation items, we analysed the survey result from  
126 different angles; intended use of data (i.e. exploratory purpose, sponsor decision making,  
127 and NDA submission; Supplementary Figure 2) and origin of a company (Japanese vs  
128 non-Japanese company; Supplementary Figure 3). While accuracy was examined in vast  
129 majority (81.3–93.8%) of the assays for sponsor decision making and NDA submission,  
130 this item was validated less frequently (60.9%) for exploratory biomarker assays.  
131 Similarly, “precision in surrogate matrix” was examined more frequently (50.0–56.3%)  
132 for the assays for sponsor decision making and NDA submission in comparison to those  
133 for exploration (30.4%). Interestingly, specificity, matrix effect, carryover, minimum  
134 required dilution, parallelism, and standard solution stability were investigated more  
135 frequently for the assays on sponsor decision making than those for exploratory purpose  
136 and NDA submission. For the other items, executing validation appeared to be  
137 independent of the intended use of data. Validation items that non-Japanese companies  
138 conducted more frequently (at least 20% differences) than Japanese companies were  
139 specificity (75.0% vs 26.5%), accuracy (100% vs 73.5%), carryover (50.0% vs 24.5%),  
140 dilution linearity/integrity (83.3% vs 63.3%), parallelism (33.3% vs 10.2%), and standard  
141 solution stability (50.0% vs 16.3%). For the other validation items, the survey results  
142 showed similar frequencies between Japanese and non-Japanese companies. We should  
143 keep in mind that the total number of answers from non-Japanese companies was only six  
144 to eight. In addition, respondents were asked if a development stage of NME affected the  
145 selection of validation items. The answer was “No” in almost all the assays (97.7%).

146 Acceptance criteria were defined in advance for the majority (76.8%) of the assays. A  
147 free-text questionnaire revealed that some respondents consulted with FDA, EMA, and  
148 MHLW guidelines on PK assay validation for defining the acceptance criteria and they  
149 did not mention white papers on biomarker assay validation at all. A part of the  
150 respondents who set the criteria used acceptance criteria lenient than those for PK assay  
151 validation.

152

## 153 **Discussion**

154 The present survey in Japan revealed the current situation of biomarker assays in clinical  
155 trials. More than half (57.7%) of the Japanese companies have conducted clinical  
156 biomarker analysis, suggesting that clinical biomarker assays have also become common  
157 in drug development in Japan. We expect this number will increase further in near future,  
158 as biomarker use has been increasing globally [12].

159 While most non-Japanese companies had SOPs on biomarker analysis, this was not the  
160 case for Japanese companies. The companies’ size and policy, and prioritized therapeutic

161 areas may partially explain this difference; however, we could not determine the  
162 reason(s). Many Japanese companies that did not have SOPs considered that it is  
163 necessary to have SOPs somewhere in the future.

164 Safety biomarkers are used for assessing patients' safety in clinical trials, and therefore,  
165 draw attention from a broad range of stake holders. In this context, a few articles state  
166 that assay validation of safety biomarkers should be more extensive than that of PD  
167 biomarkers [6,13]. The present survey revealed additional differences in the  
168 characteristics between PD biomarkers and safety biomarkers; PD biomarkers were  
169 analysed much more frequently than safety biomarkers.

170 For an intended use of biomarker data, a third of the assays were conducted to support the  
171 regulatory review of NMEs. In other words, assays for exploration and sponsor decision  
172 making were in majority, suggesting that it is prudent to discuss the level of validation of  
173 the assays for exploration and sponsor decision making carefully. In 2006, Lee et al.  
174 proposed fit-for-purpose approach for the biomarker assay validation (such as exploratory  
175 assay validation and advanced assay validation) wherein rigor of validation depends on  
176 the intended use of biomarker data [5]. This approach assists the pharmaceutical  
177 companies conserving resources for the exploration of biomarkers and sponsor decision  
178 making. Fit-for-purpose approach is supported by FDA guidance 2018 [14]. While the  
179 guidance requires full validation of biomarker assays to support the regulatory decision  
180 making, it allows the industry to decide the extent of assay validation for exploratory  
181 assays.

182 According to the survey results, the majority of biomarker assays consisted of three types  
183 as follows; a) chromatographic assays to be developed de novo, b) ligand binding assays  
184 to be developed de novo, and c) ligand binding assays using commercial kits. This  
185 finding was important, as points to consider in assay validation depends on methodology.  
186 In addition, usage of commercial kits needs some consideration [15]. For example, it is a  
187 good practice to confirm that a commercial kit measures an analyte of interest by  
188 experimentation. It is recommended to focus on the above-mentioned three types of  
189 biomarker assays during initial discussion on biomarker assay validation. Henceforth, it  
190 will be necessary to discuss other methodologies (including flow cytometry and  
191 polymerase chain reaction assays) and newly developed technologies (including large  
192 molecule analysis by LC-MS).

193 The present survey revealed that surrogate matrix was used as the matrix for the  
194 preparation of samples at known concentration in some assays. This appears reasonable;  
195 if endogenous analyte concentration is detectable in the same biological matrix as the  
196 study samples, use of surrogate matrix is a common strategy to prepare calibration  
197 samples without interference from an endogenous analyte [5]. This strategy is one of the  
198 options for chromatographic assays (i.e. in the other option, calibration samples can be  
199 prepared by spiking stable isotope-labelled analytes into the same biological matrix as the  
200 study samples) [16]. However, use of surrogate matrix is essentially the only option for

201 ligand binding assays [17]. In addition, the present survey revealed that most biomarker  
202 assays are ligand binding assays.

203 For validation items, GCC reported the survey results collected from European and North  
204 American CROs in 2012 [9]. Respondents were asked whether they included calibration,  
205 precision and accuracy, selectivity/specificity, parallelism, storage stability, sensitivity,  
206 linearity of dilution, recovery when they validated ligand binding assays, and small  
207 molecule assays. When we compare the GCC survey results with the present survey  
208 results, we should be careful about the known and unknown differences in the methods of  
209 the two surveys. Validation items that were more frequently reported to be examined in  
210 the present survey in comparison to the GCC survey were LLOQ for ligand binding  
211 assays (90.6% vs 56–60%) and chromatographic assays (100% vs 60–75%), and dilution  
212 linearity for ligand binding assays (83.3% vs 44–60%). Validation items that were less  
213 frequently reported to be examined in the present survey than the GCC survey was  
214 parallelism for ligand binding assays (16.7% vs 60–67%) and chromatographic assays  
215 (0% vs 25–30%).

216 Lee et al. suggested to estimate LLOQ for exploratory assay validation and establish  
217 LLOQ for advanced assay validation [5], whereas Cummings et al. and Chau et al.  
218 suggested to include LLOQ for all the assays [18,19]. Lee et al. suggested to include  
219 dilution linearity and parallelism for both assay validation [5], whereas Cummings et al.  
220 and Chau et al. suggested to include dilution linearity and parallelism only for definitive  
221 and relative quantitative assays [18,19]. The differences among the three articles might be  
222 linked to the differences between the present survey and the GCC survey. It is noteworthy  
223 that recent discussions in workshops and points to consider document emphasize the  
224 importance of parallelism [8,11]. We should keep in mind that it is not always possible to  
225 include parallelism in assay validation due to limited sample availability.

226 In the present survey, development stage did not affect the validation items. This is in line  
227 with the EBF white paper [6].

228 Respondents in the present survey did not mention about white papers in biomarker assay  
229 validations, when they were asked about documents they consult with. Those in the GCC  
230 survey mentioned regulatory guidelines on PK assay validation as well as white papers  
231 on biomarker assay validation by Lee et al. [5], Nowatzke et al. [20], Valentin et al. [17],  
232 Cummings et al. [18], and Chau et al. [19]. Considering the differences between PK  
233 assays and biomarker assays, it is good to have points to consider or regulatory  
234 documents they can rely on about biomarker assays also in Japan.

235

## 236 **Conclusions**

- 237 ● The present survey in Japan revealed that biomarker assays during clinical trials have  
238 become common in drug development, and approximately 30% of the assays are for  
239 regulatory decision making.
- 240 ● It is recommended to assume that the majority of biomarker assays consisted of three

241 types as follows; a) chromatographic assays to be developed de novo, b) ligand  
242 binding assays to be developed de novo, and c) ligand binding assays using  
243 commercial kits. In future, it will be necessary to discuss other methodologies and  
244 newly developed technologies.

245 ● When the respondents designate acceptance criteria, they consult PK assay  
246 guidelines, and not biomarker assay white papers. FDA guidance 2018, which was  
247 issued after the present survey, provided only limited recommendations on biomarker  
248 assays. It is important to have points to consider or regulatory documents, which can  
249 be embraced by the Japanese bioanalysis community.

250 ● While we found that parallelism was not tested very often in Japan, parallelism was  
251 conducted in most (60–67%) of the ligand binding assays in North American and  
252 European CROs [9]. We should discuss the necessity of parallelism in future. We  
253 hope that this survey will facilitate discussion on biomarker assay validation, and  
254 would therefore promote the usage of biomarkers in drug development.

255

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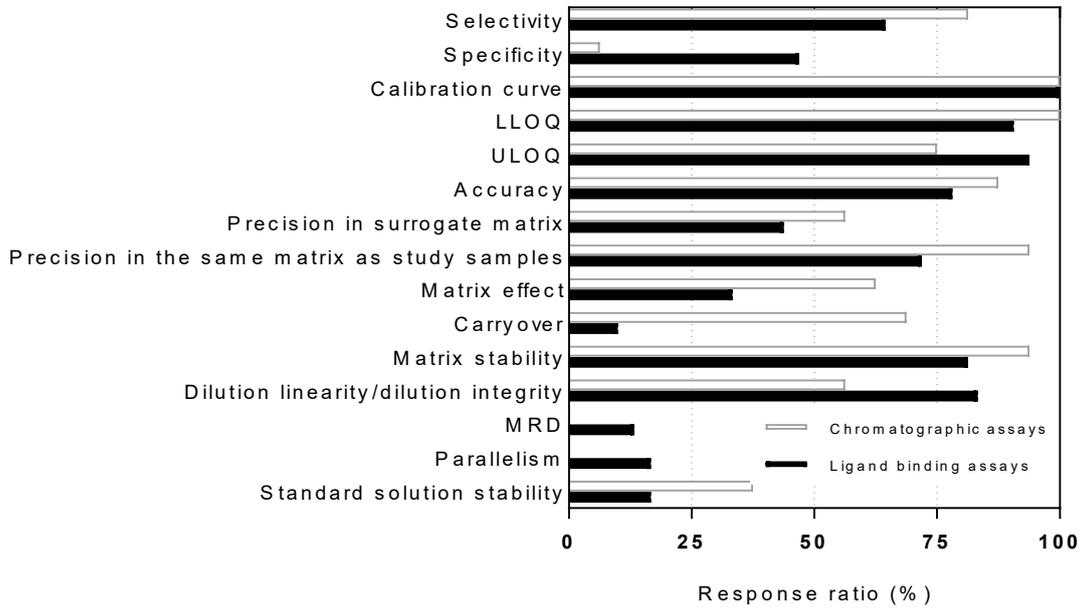
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- 317
- 318 Reference annotations (Authors should highlight references that are of particular  
319 significance to the subject under discussion as “\* of interest” or “\*\* of considerable  
320 interest”, and provide a brief (1–2 line) synopsis.)

321 \*\*  
322 5 Lee 2006  
323 Fundamental concept of biomarker assay validation was proposed.  
324  
325 \*  
326 11 Critical Path Institute 2017  
327 Fit-for-purpose acceptance criteria were proposed.  
328  
329 14 FDA 2018  
330 This is the first regulatory document for biomarker assay validation.  
331  
332

333 **Supplementary information**

334

335 Supplementary Figure 1. Test items examined during validation of ligand binding assays  
 336 (black bars) and chromatographic assays (white bars). MRD: Minimum required dilution.

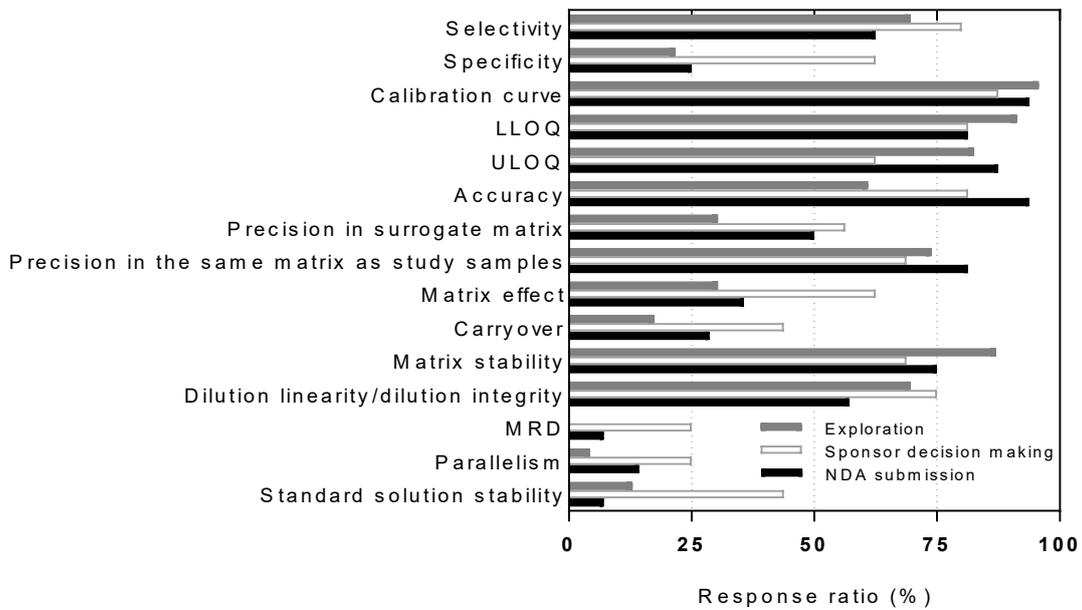


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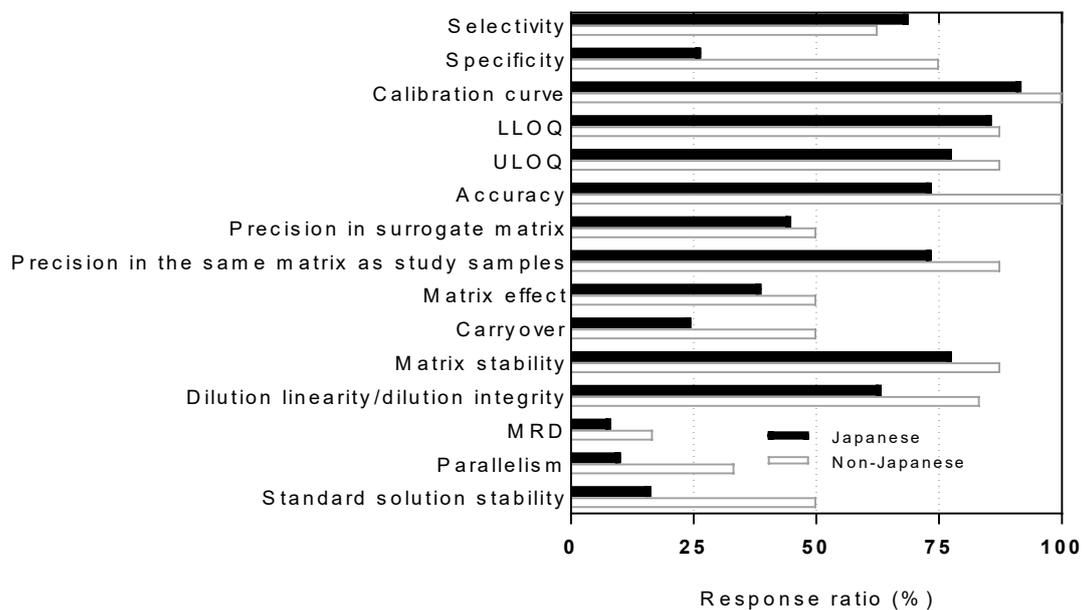
340 Supplementary Figure 2. Effect of data usage on test items examined during assay  
 341 validation. Exploration: grey bars, sponsor decision making: white bars, NDA  
 342 submission: grey bars. MRD: Minimum required dilution.



343

344

345 Supplementary Figure 3. Effect of an origin of a company on test items examined during  
346 assay validation. Japanese companies: black bars, non-Japanese companies: white bars.  
347 MRD: Minimum required dilution.



348  
349