Developability assessment for nonspecificity and polyspecificity in high-throughput bead-based assays in microplates

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Abstract

Testing for non- and polyspecificity of biologics is an increasingly important aspect for drug discovery and development because there is increasing evidence that it is strongly related to success in clinical testing. Surface hydrophobicity and positive surface patches have been identified as the two most unfavorable molecular features. Although this is becoming common knowledge, the industry is lacking fast and standardized methods to screen thousands of candidates. Instead, many drug developers use tedious in-house ELISA assays with poorly defined components or slow and labor-intensive chromatographic methods.

PAIA is addressing these issues by proposing a set of high throughput assays that use the same easy-to-automate microplate-based workflow. The assays use chemically defined beads to detect the binding of drug candidates to hydrophobic (HIC-like) and negatively charged (CEX-like) surfaces to assess nonspecific binding and ovalbumin (OVA) loaded beads as a predictor for polyspecificity as well as beads with Heparin (HEP) which has been reported to be an indicator for pinocytosis. The assays are entirely performed in special PAIA microplates and provide results for up to 384 samples in less than 45 minutes. They assays only require a powerful microplate shaker and a fluorescence plate reader with bottom read capability.

Assay principle

Figure 1. Assay workflow on PAIA microplates
The whole assay is performed in the 384-well PAIAplate which is filled with dried capture beads [1]. After addition of the fluorescent nanobody and sample [2], the plate is shaken for 30 min [3] and the beads are settled on the bottom and thereby out of the measurement area [4]. The plates can be read on a fluorescence plate reader with bottom reading [5]. The reading only detects the fluorescence in solution and therefore binders will show low fluorescence values.

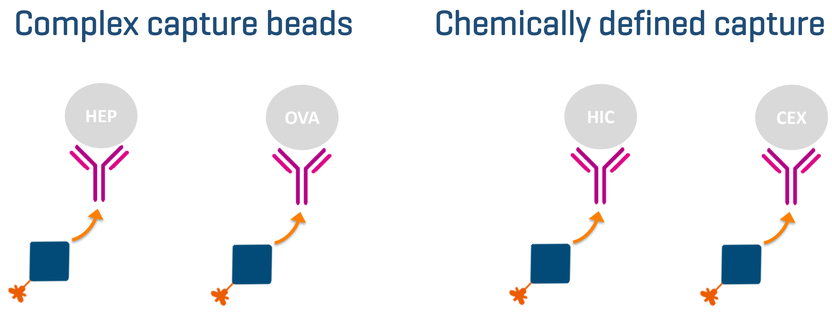

Figure 2. Assay components in the non- and polyspecificity assays (sandwich format)
The different capture beads are depicted as grey circles, to which the antibodies can bind. The binding is detected by a fluorescence-labeled Nanobody (blue squares) with high affinity for the Fc domain of the antibody. The samples only need to be diluted in water by a factor of 10 or higher. Supernatant samples can be run using the same sample preparation.

Materials and methods

We selected a panel of 24 well characterized clinical stage antibodies with different properties in the assay clusters that are most related to clinical success and failure (see Table, data taken from Jain et al. 2023 [1]). All antibodies were analyzed with the PAIA assays and the results were compared with published data (HIC and OVA) as well as in-house chromatography results (CEX). In addition, we tested the antibodies in different concentrations to determine the assay range and we tested the feasibility of all three assays for measuring non-purified samples directly (results not shown).

In brief, the samples were diluted in DI water to a concentration of 10 µg/mL and 20 µL of the sample was dispensed into the wells of the 384-well PAIA plate. 40µL of the fluorescence-labeled Nanobody was added, and the microplate was shaken at 2200 rpm on an orbital shaker for 30 minutes. Afterwards the plate was quickly centrifuged at 500 xg and read on a plate reader in bottom read mode at 640/670nm.

Antibody name	Cluster 3 HIC, SMAC, SGAC No. of red flags	Cluster 6 ELISA, BVP etc. No. of red flags	Cluster 7 PSR, ACSINS, CSI, CIC, FcRn No. of red flags
adalimumab	0	0	0
belimumab	0	2	0
bevacizumab	1	0	0
blosozumab	0	2	1
bococizumab	1	2	5
brentuximab	0	1	0
cetuximab	0	0	0
cixutumumab	2	2	4
codrituzumab	0	2	0
denosumab	0	2	1
dupilumab	0	2	1
emibetuzumab	1	2	3
foralumab	1	2	2
ganitumab	0	1	2
ixekizumab	0	2	3
lenzimumab	1	2	5
mepolizumab	0	0	0
ofatumumab	0	0	0
omalizumab	0	0	0
panitumumab	0	0	0
pertuzumab	0	0	0
rituximab	0	0	2
simtuzumab	0	2	1
trastuzumab	0	0	0

Hydrophobic interaction assay (HIC)

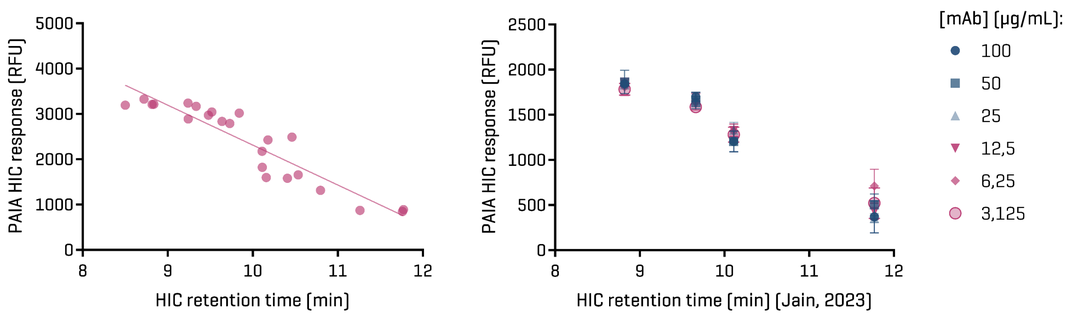

Figure 3. Comparison of HIC assay results with published retention time data
Left: the fluorescence intensities for all 24 Mabs were plotted against published HIC data (from Jain et al. 2023). The correlation is excellent and all hydrophobic Mabs are successfully identified as high binders (with low fluorescence signals). Right: four Mabs were analyzed at different concentrations showing that the assay is concentration-independent in this range.

Cation exchange assay (CEX)

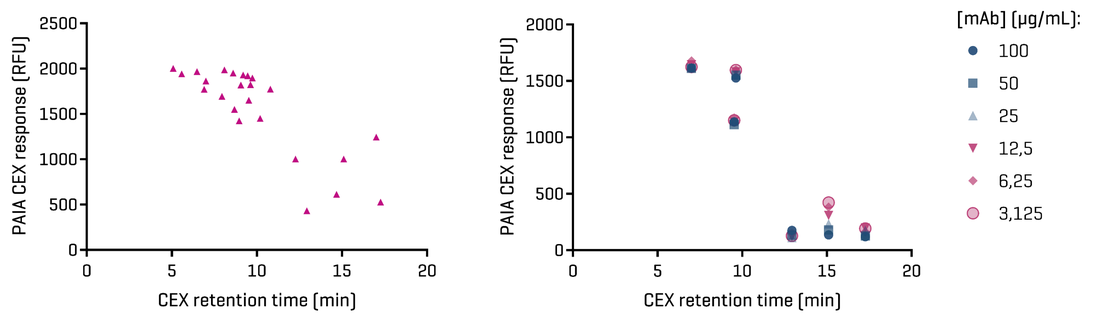

Figure 4. Comparison of CEX assay results with in-house CEX data
Left: the fluorescence intensities for all 24 Mabs were plotted against in-house CEX chromatography results. Right: six Mabs were analyzed at different concentrations showing that the assay is concentration-independent in the range between 3 and 100 µg/mL. Samples therefore do not have to be normalized for titer before screening.

Ovalbumin binding assay (OVA)

Ovalbumin was recently proposed as a reagent to test for polyspecificity because it is a complex protein with low pI and hydrophobic patches (Makowski et al 2021 [2]). In addition, ovalbumin has multiple PTMs like glycosylation, acetylation and phosphorylation, which can also potentially interact with the antibodies.

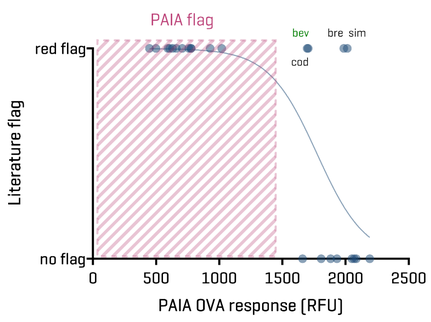

Figure 5. Comparison of Ovalbumin binding results with PSR flags from literature
The assay results for all 24 Mabs reach from less than 500 to more than 2000 counts. All Mabs with intensities lower than 1500 counts (the threshold) are considered ovalbumin binders. These molecules are all red-flagged in Jain et al. which means that they have liabilities in the assay clusters listed in Table 1. The Mabs with high intensities are non-binders and this corresponds equally well with literature.

Heparin binding assay (HEP)

Heparin chromatography is used as a surrogate for the highly negatively charged glycocalyx on endothelial cells, which are involved in nonspecific clearance (Kraft et al., 2020 [3]).
We used Heparin-loaded beads to study the Heparin binding of our Mab panel at defined buffer conditions and salt concentrations.

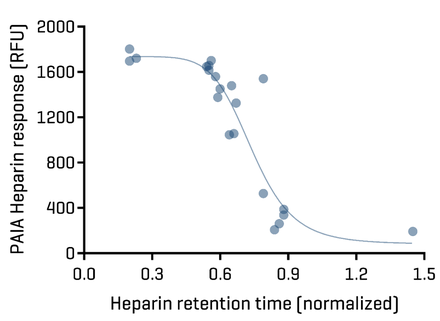

Figure 6. Comparison of the Heparin binding assay results with published data
The heparin binding assay correctly identifies the non-binders (high fluorescence intensities) and the very strong binder Lenzilumab (low fluorescence). The values of the remaining Mabs are correlating in a quasi-linear fashion with the published Heparin chromatography data.

1: Tushar Jain, Todd Boland & Maximiliano Vásquez (2023) Identifying developability risks for clinical progression of antibodies using high-throughput in vitro and in silico approaches, mAbs, 15:1, DOI: 10.1080/19420862.2023.2200540
2. Emily K. Makowski, Lina Wu, Alec A. Desai & Peter M. Tessier (2021) Highly sensitive detection of antibody nonspecific interactions using flow cytometry, mAbs, 13:1, DOI: 10.1080/19420862.2021.1951426
3. Thomas E. Kraft, Wolfgang F. Richter, Thomas Emrich, Alexander Knaupp, Michaela Schuster, Andreas Wolfert & Hubert Kettenberger (2020) Heparin chromatography as an in vitro predictor for antibody clearance rate through pinocytosis, mAbs, 12:1, DOI: 10.1080/19420862.2019.1683432