Novartis Pharma AG
TRD Biologics

Development of a new cell adhesion bioassay format

Frieder Kröner, PhD
CASSS Bioassays 2021
April 19-23, 2021

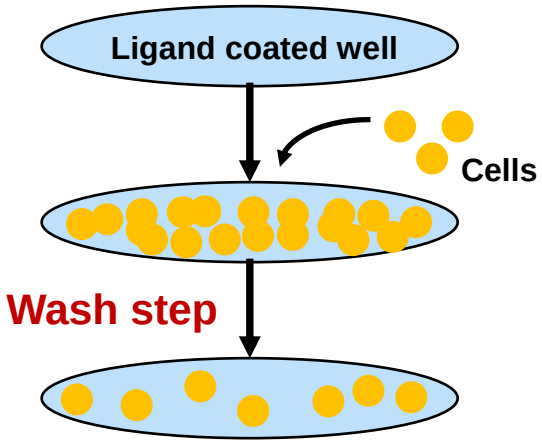
NOVARTIS | Reimagining Medicine

Introduction

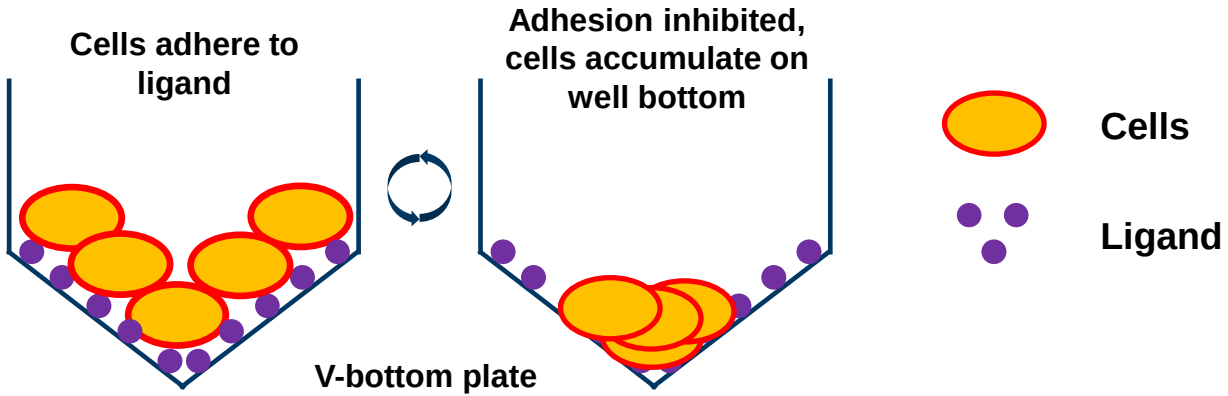
Biologic drug with MoA = inhibition of cell-cell adhesion; specific target on specific cell type.

Classic cell adhesion assays require separation of non-adherent cells by manual plate washing / inversion / tapping.

This causes assay variability as operator dependent and difficult to define / control, especially when adhesion is weak and / or involves cell rolling.



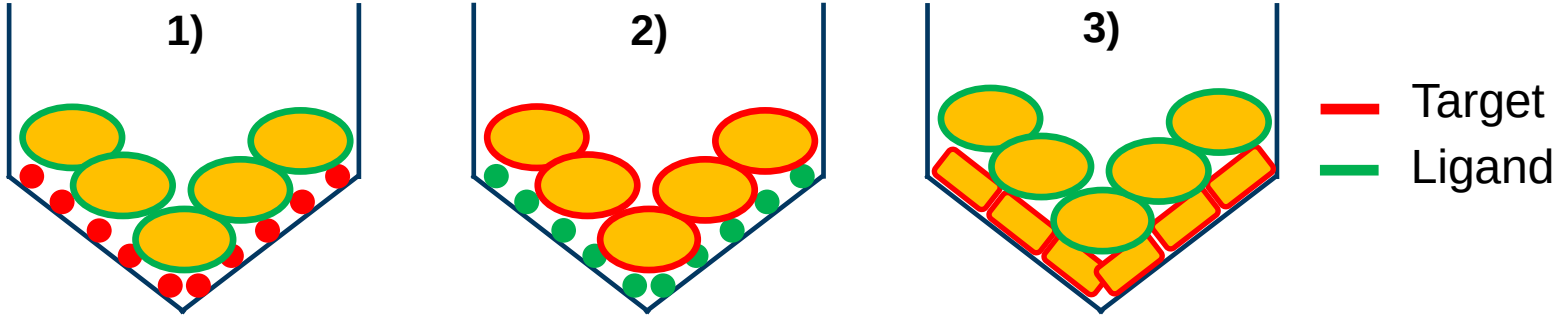
V-plate cell adhesion assay for HTS



(Weetall et al. 2001)
A Homogeneous Fluorometric Assay for Measuring Cell Adhesion to Immobilized Ligand Using V-Well Microtiter Plates, Analytical Biochemistry, Volume 293, Issue 2, 2001

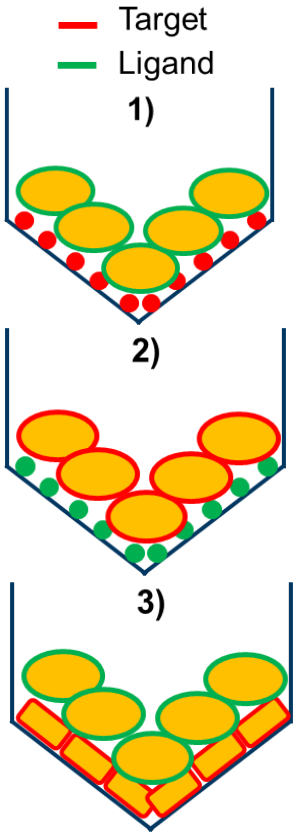
- V-bottom plates centrifuged to separate adherent from non-adherent cells.
- Centrifugation can be standardized and is not operator dependent.
- Objective: convert HTS assay (designed to qualitatively identify “hits”) into a quantitative relative potency bioassay for QC testing.

Evaluation of three different formats



Drug Target	**Coated to the plate**	**Recombinantly expressed by cells**	**Recombinantly expressed by cells "coated" to the plate**
Ligand of drug target	**Endogenously expressed by cells**	**Coated to the plate**	**Endogenously expressed by cells**

Evaluation of three different formats



In all three setups a dose-response was obtained, but:

1) Specific cell line expressing ligand needed since ligand requires a specific post-translational modification; variable expression of modified ligand led to low robustness.

2) Better robustness than 1) due to coating of recombinant ligand with controllable quality and transfected cells stably expressing target.

3) Not developed further due to complexity (2 cell lines) and since format 2) was found suitable.

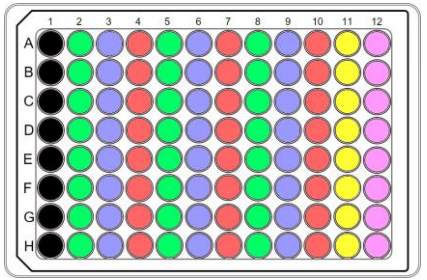
Coating, blocking, sample & cell prep.

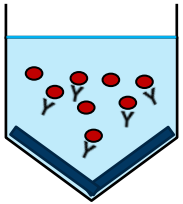
1. Ligand of drug target coated on V-bottom plate, subsequently blocked with BSA.

2. Samples diluted in assay medium, added to the plate and temperature equilibrated. Samples (S1, S2), reference, positive-, negative-, **blank**-control added.

3. Cells stably transfected with drug target are fluorescently labeled using calcein and added to plate.

Ligand coated
V-bottom 96-well MTP





Cells ● + Y Inhibitor

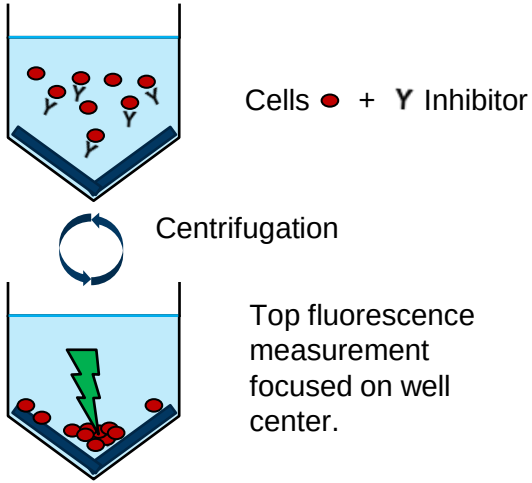
NOVARTIS | Reimagining Medicine

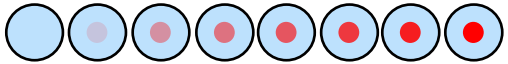
Adhesion, centrifugation, readout

4. Cells incubated with samples on plate and centrifuged at (300 g, 10 min).

5. Fluorescence measurement from top focused on the cells accumulated in the bottom / center of V-bottom plate wells.

Note: the assay (w/o coating) is performed in ~ half a day.

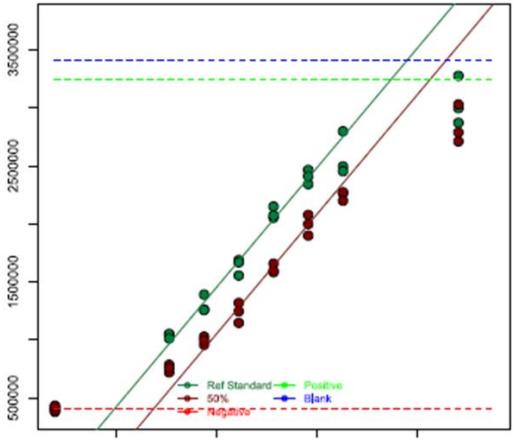




Cell pellet formed due to increasing drug dose (left to right)

Data analysis & validation

E.g. 50% sample (curve shifted right)



- Serial dilutions with extended asymptotes (large first and last dilution step).
- Relative potency of the sample is determined against the reference standard.
- Evaluated 4P fit and parallel line analysis (PLA) – PLA gave better accuracy.
- SSTs on regression / fit, parallelism, signal:noise and relative confidence limits of potency estimate.

Method fully validated for routine QC testing based on ICH Q2B.

E.g.: Range 50-150%, accuracy 89-98%, repeatability GRSD = 5%, precision GRSD = 10%, reproducible between labs, selective vs. unrelated drugs.

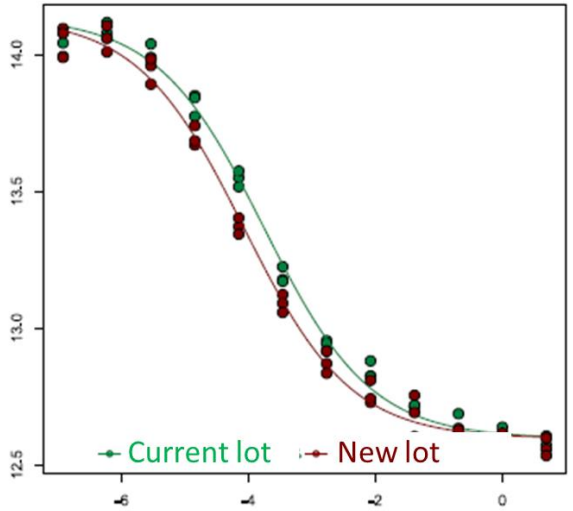
Assay robustness, critical parameters

- Critical parameters identified during method development and robustness DoE.
- Parameters critical to cell-based bioassays in general, e.g. cell cultivation (as split schedule, max passage number,...), critical reagents,...
- Critical parameters specific to the assay format:
  - V-bottom plates
  - Centrifuge type
  - Fluorescence reader & settings

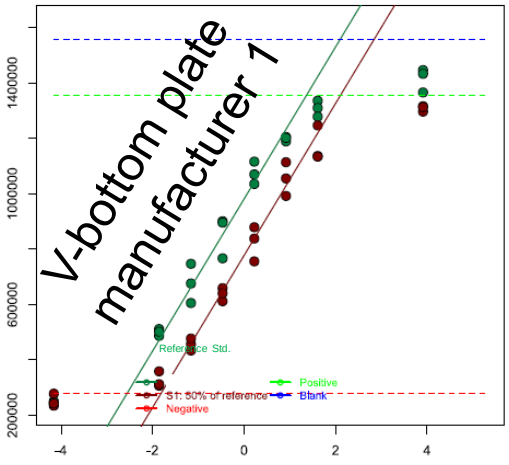
Coated ligand

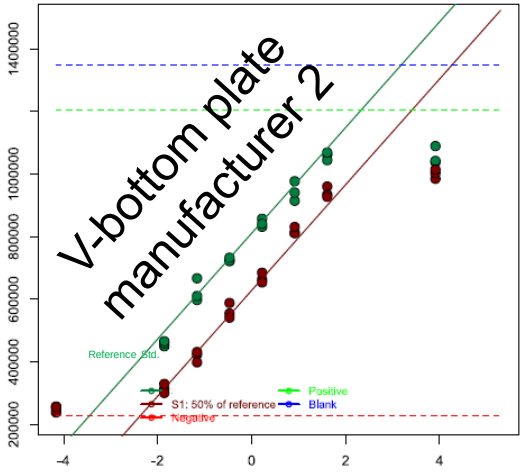
Activity of coated ligand strongly dependent on a post translational modification:

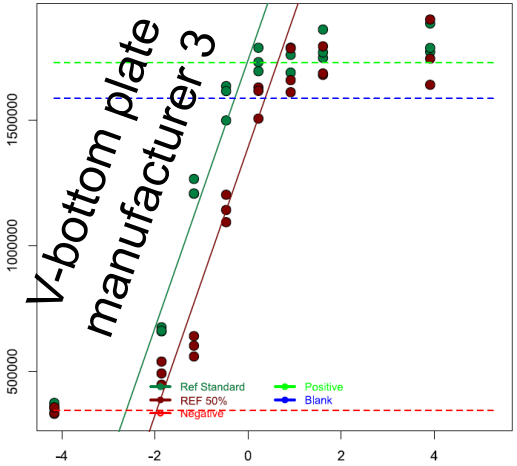
- Activity of each new lot is controlled (titration of coated drug target ligand).
- Since limited sources exist, extra measures taken to ensure business continuity during product life cycle (supplier QAA, stocks, long-term stability,...).



V-bottom plate





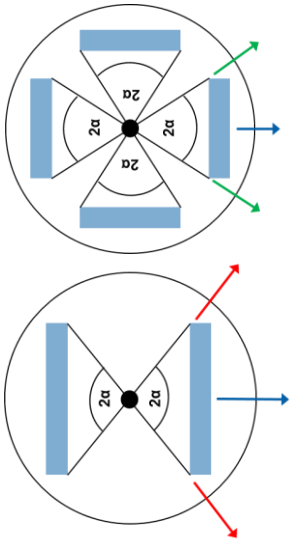


- V-plate surface and dimensions (e.g. V-angle) may vary between manufacturers and plate types.
- Manufacturer 1 and 2 interchangeable; manufacturer 3 not suitable.

NOVARTIS | Reimagining Medicine

Centrifuge type

Top view on centrifuges



4-slot rotor, vertical plate orientation

rows A to H

1364723	1262687	1268716	1258544	1213090	1222897	1231999	1264740	1252951	1203557	1267657	805912
1540231	1441188	1401916	1393453	1397929	1380147	1399104	1421666	1468669	1381563	1452293	1318567
1575590	1453341	1458094	1490719	1466925	1478866	1484059	1481015	1515787	1398484	1450718	1357970
1572110	1458215	1441571	1528636	1481319	1461983	1521217	1552112	1504681	1433196	1496398	1411727
1544284	1420857	1415800	1542492	1566430	1494692	1523098	1518422	1542591	1422005	1488520	1404225
1455807	1357274	1372331	1409034	1393361	1393582	1436260	1424895	1473284	1387024	1386695	1356235
1401931	1307911	1316816	1344434	1312415	1292531	1323346	1384833	1365332	1294536	1339052	1274536
1179238	1146472	1138725	1122122	1137439	1092984	1164594	1139489	1206899	1121056	1154957	1154392

Columns 1-12

2-slot rotor, horizontal plate orientation

rows A to H

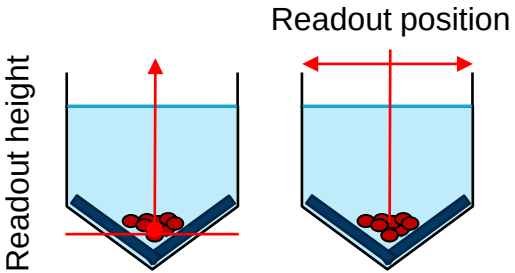
65071	683314	1184606	1372878	1492438	1483290	1491686	1404806	1268777	935080	623240	150228
78287	862951	1248849	1415021	1389980	1490201	1450666	1360824	1242371	991049	698334	164696
102857	936438	1296565	1351459	1500756	1510254	1487542	1409769	1257181	1021781	752478	173227
129065	810040	1309363	1454526	1543337	1550643	1499849	1397256	1313632	1014191	735488	189511
115278	938014	1300689	1500348	1498202	1512513	1513658	1523592	1413280	1078349	795466	194062
164399	942522	1341525	1474217	1491202	1503360	1462487	1391726	1311145	1031767	731880	205237
232924	1129235	1352128	1423033	1494659	1454158	1427953	1344487	1288023	1012703	746260	222413
239636	981221	1262293	1304073	1363832	1315488	1352865	1257234	1181080	929125	752202	235452

Columns 1-12

Fluorescence intensity per plate well

- Experiment: Positive control in all plate positions.
- Large vertical 4 slot rotor: centrifugal force angle only varies minimally across the plate.
- Small horizontal 2-slot rotor: centrifugal force angle varies strongly across the plate.

Reader settings



Reader with properly calibrated X / Y axis (signal intensity per well)

rows A to H

1364723	1262687	1268716	1258544	1213090	1222897	1231999	1264740	1252951	1203557	1267657	805912
1540231	1441188	1401916	1393453	1397929	1380147	1399104	1421666	1468669	1381563	1452293	1318567
1575590	1453341	1458094	1490719	1466925	1478866	1484059	1481015	1515787	1398484	1450718	1357970
1572110	1458215	1441571	1528636	1481319	1461983	1521217	1552112	1504681	1433196	1496398	1411727
1544284	1420857	1415800	1542492	1566430	1494692	1523098	1518422	1542591	1422005	1488520	1404225
1455807	1357274	1372331	1409034	1393361	1393582	1436260	1424895	1473284	1387024	1386695	1356235
1401931	1307911	1316816	1344434	1312415	1292531	1323346	1384833	1365332	1294536	1339052	1274536
1179238	1146472	1138725	1122122	1137439	1092984	1164594	1139489	1206899	1121056	1154957	1154392

Columns 1 to 12

Reader with wrongly calibrated X / Y axis (signal intensity per well)

rows A to H

1,100,084	1,158,596	1,106,960	1,190,604	1,180,180	1,230,322	1,223,437	1,058,896	1,207,877	1,256,781	1,091,762	1,154,789
1,196,771	1,317,214	1,337,962	1,396,310	1,316,048	1,368,763	1,458,955	1,284,801	1,460,185	1,535,368	1,299,490	1,287,046
1,351,847	1,587,723	1,626,737	1,627,064	1,609,212	1,540,048	1,534,185	1,595,725	1,601,864	1,694,969	1,593,673	1,514,574
1,629,625	1,781,716	1,678,914	1,813,873	1,887,719	1,861,617	1,853,930	1,760,358	1,860,690	2,048,728	1,902,117	1,777,769
1,871,003	2,273,210	2,142,310	2,226,079	2,299,912	2,280,947	2,343,380	2,258,971	2,338,461	2,262,418	2,109,679	2,002,834
2,117,236	2,453,942	2,196,683	2,477,711	2,396,371	2,390,404	2,457,857	2,436,475	2,498,293	2,553,027	2,323,862	2,190,677
2,144,728	2,459,259	2,287,167	2,310,485	2,315,175	2,381,601	2,308,939	2,292,413	2,448,004	2,395,029	2,215,231	2,139,536
1,963,461	2,025,466	1,960,501	2,069,423	2,009,248	2,042,397	1,896,708	1,930,031	2,088,910	1,986,850	1,866,364	1,908,262

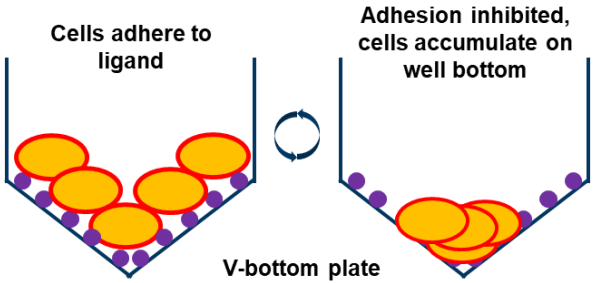
Columns 1 to 12

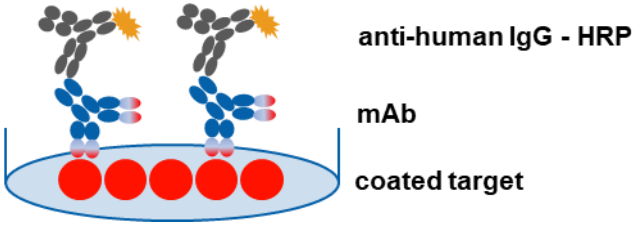
Pos. control all over the plate

Reader optics need to be correctly focused on the center of the V-shaped well

- Readout position & plate dimensions have to be accurately defined.
- X/Y-axis of the reader might need re-calibration if a signal drift is recognized.

New assay format - implementation



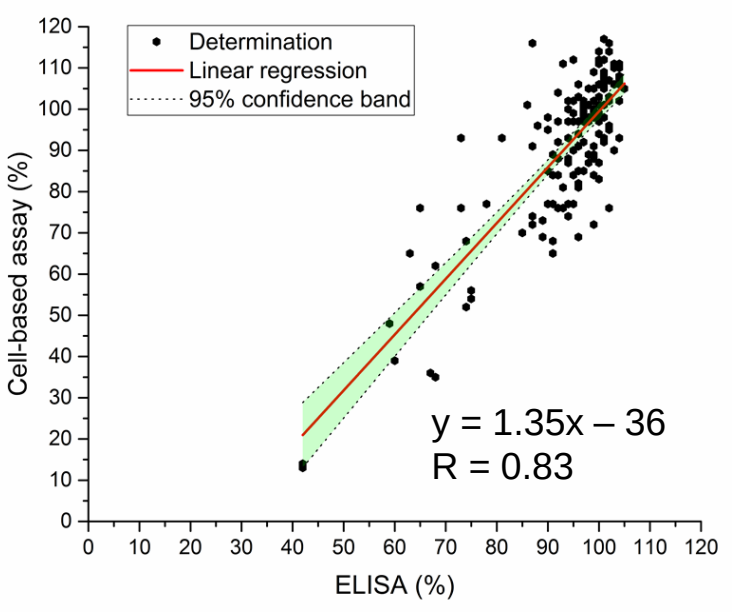


New adhesion assay format compared to target binding ELISA

- DS / DP release and stability samples, drug variants (charge, size, etc.) and degradation products analyzed in both assays

Comparison to ELISA

- DS / DP release and stability samples were analyzed in both assays.
- Correlation observed - slope and y-intercept indicate that decrease in potency is recognized more sensitively by the cell adhesion bioassay.
- ELISA was replaced by cell adhesion bioassay.



NOVARTIS | Reimagining Medicine

Summary

- Cell adhesion assay originally developed for HTS was repurposed and redeveloped as a CMC relative potency bioassay.
- New format successfully validated and implemented for routine testing replacing a target binding ELISA, as superior in detecting potency changes.
- New format was robust. Specific parameters (e.g. centrifuge, V-bottom plates reader settings) identified and controlled.
  - Main sources of variability in classic adhesion assays not present in new format, resulting in better assay control & robustness.
- May be generally applicable to drugs with cell adhesion as MoA.

Thank you

Acknowledgments:

Jeremy Grunenwald
Carla Schmidt

Izabela Slupska-Muanza
Denise Kubon
Julien Ribstein
Keliann Lesault
Stephanie Larti
Melanie Juffa

Oliver Anderka
Tim Kees

Thomas Millward
Emilie Ferrier
Steffen Pahlich
Fabian Bickel
Dirk Chelius
Michael Safinowski
Katja Baer

NOVARTIS | Reimagining Medicine