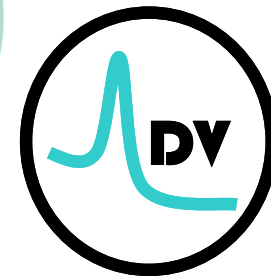*Analytical Detection and Characterization of Host Cell Proteins*

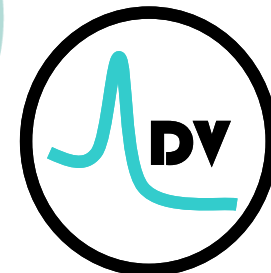*Stefanie Fas, PhD*
Associate Principal Scientist
BMSC, Analytical Development & Validation
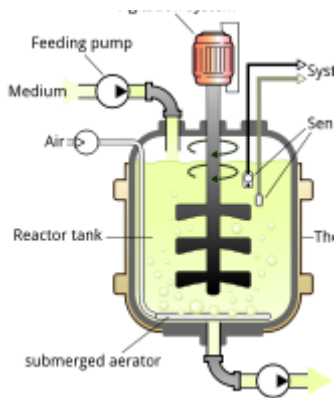*MSD*

Overview

- **Introduction HCP, detection by ELISA**

- Comparison of commercially available CHO kits

- Assessment of suitability of a commercial kit

- Conformational coverage determination
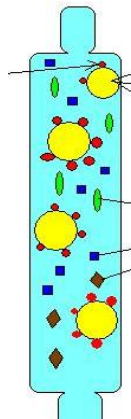
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Challenges for HCP assays

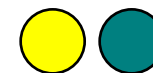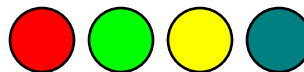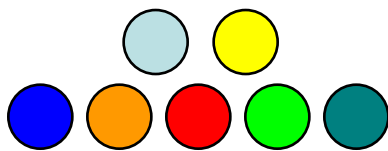Manufacturing   Purification   Drug substance (DS)
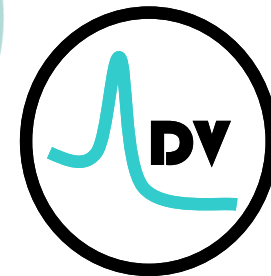

Host cell Proteins (HCP)

- Many different HCP and high diversity
- Different amounts
- Sensitive assay (ICH Q6B)

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MSD
Be well

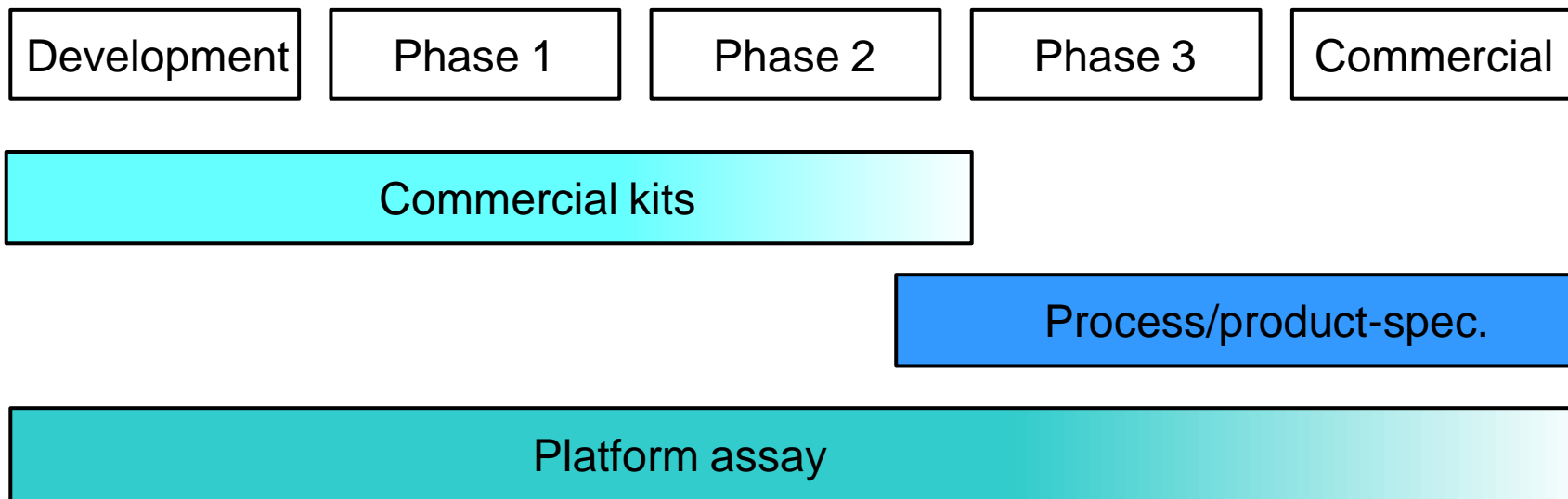# HCP determination

- ELISA is current method of choice
- Quantitation (high sensitivity) possible
- Complemented by other methods (e.g. MS)

Detection Ab

HRP

Substrate reaction

TMB

HCP

Capture Ab

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HCP ELISA

| Development | Phase 1 | Phase 2 | Phase 3 | Commercial |

Commercial kits

Process/product-spec.

Platform assay
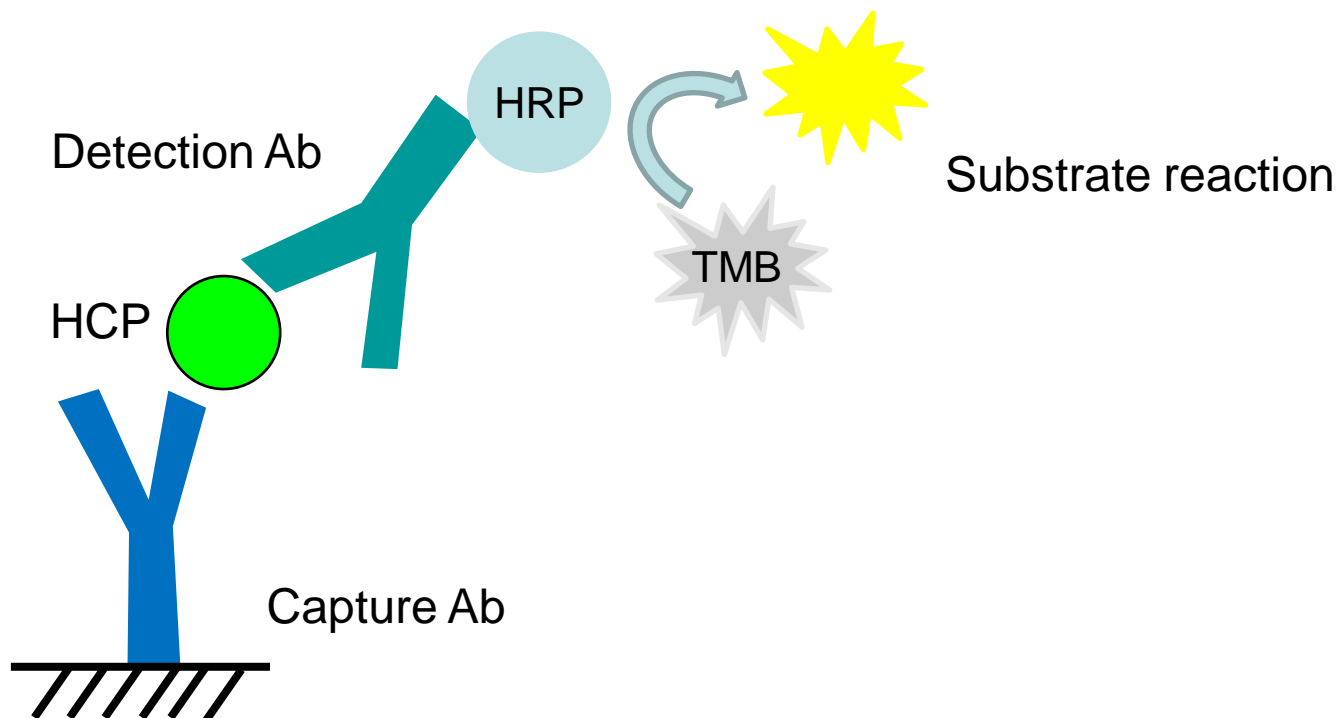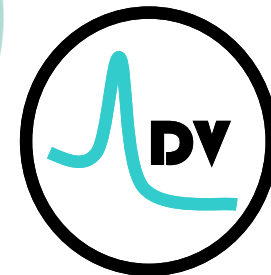
- Commercial kits
  - Readily available
  - Dependence on supplier
  - Not process-specific
  - Different suppliers available

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Overview

- Introduction HCP, Detection by ELISA

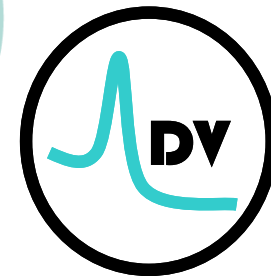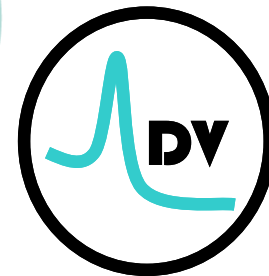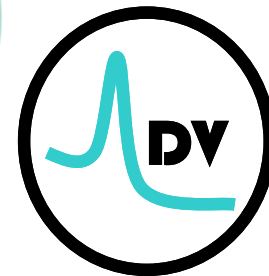- **Comparison of commercially available kits**

- Assessment of suitability of a commercial kit

- Conformational coverage determination

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Comparison of commercially available kits

- Tested kits:
    - Cygnus 2G (CM015)
    - Cygnus 3G (F550)
    - Krishgen BioSystems (KBBP03)
    - Alpha Diagnostic International (800-140-CHO)
    - Array Bridge (AB00101)
    - 4x Biogenes (Enhanced generic CHO/360-HCP ELISA kits A-D)
- Comparison with process-specific CHO HCP ELISA

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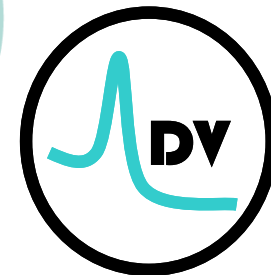# Comparison of commercially available kits

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  - Alpha Diagnostic International (800-140-CHO)
  - Array Bridge (AB00101)
  - Biogenes (Enhanced generic CHO/360-HCP ELISA kits A-D)
- Comparison with process-specific CHO HCP ELISA

Comparison of different commercially available CHO kits with a product/process-specific HCP EIA

- 1) Amount HCP in 3 DS (therapeutic antibodies, produced by CHO)
- 2) Recovery of 3 product-specific Mock CHO HCP (indication for coverage)
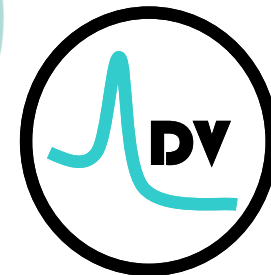- 3) Assess dilutional linearity (DS)

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HCP content of DS using different kits

	HCP content (ppm)		
	DS1	DS2	DS3
Cygnus 2G	0*	0*	0.3
Cygnus 3G	1.8	9.4	0.9
Alpha Diagnostic	2.3	9.6	1.4
Array Bridge	15.6	19.5	10.8
Krishgen	0*	0*	2.2
Biogenes A	2.3	1.4	0.4
Biogenes B	0.7	1.3	0.2
Biogenes C	1.8	1.0	0.5
Biogenes D	1.5	1.8	0.5
DS2-spec	2.8	2.7	1.7
Range	0-16	0-20	0.2-11
*: below LOQ			

- Measured HCP content is dependent on assay

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HCP content of DS using different kits

	HCP content (ppm)		
	DS1	DS2	DS3
Cygnus 2G	0*	0*	0.3
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Biogenes D	1.5	1.8	0.5
DS2-spec	2.8	2.7	1.7
Range	0-16	0-20	0.2-11
*: below LOQ			

- Measured HCP content is dependent on assay

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Recognition of Mock HCP in kits and process-specific HCP EIA

- Different recognition patterns

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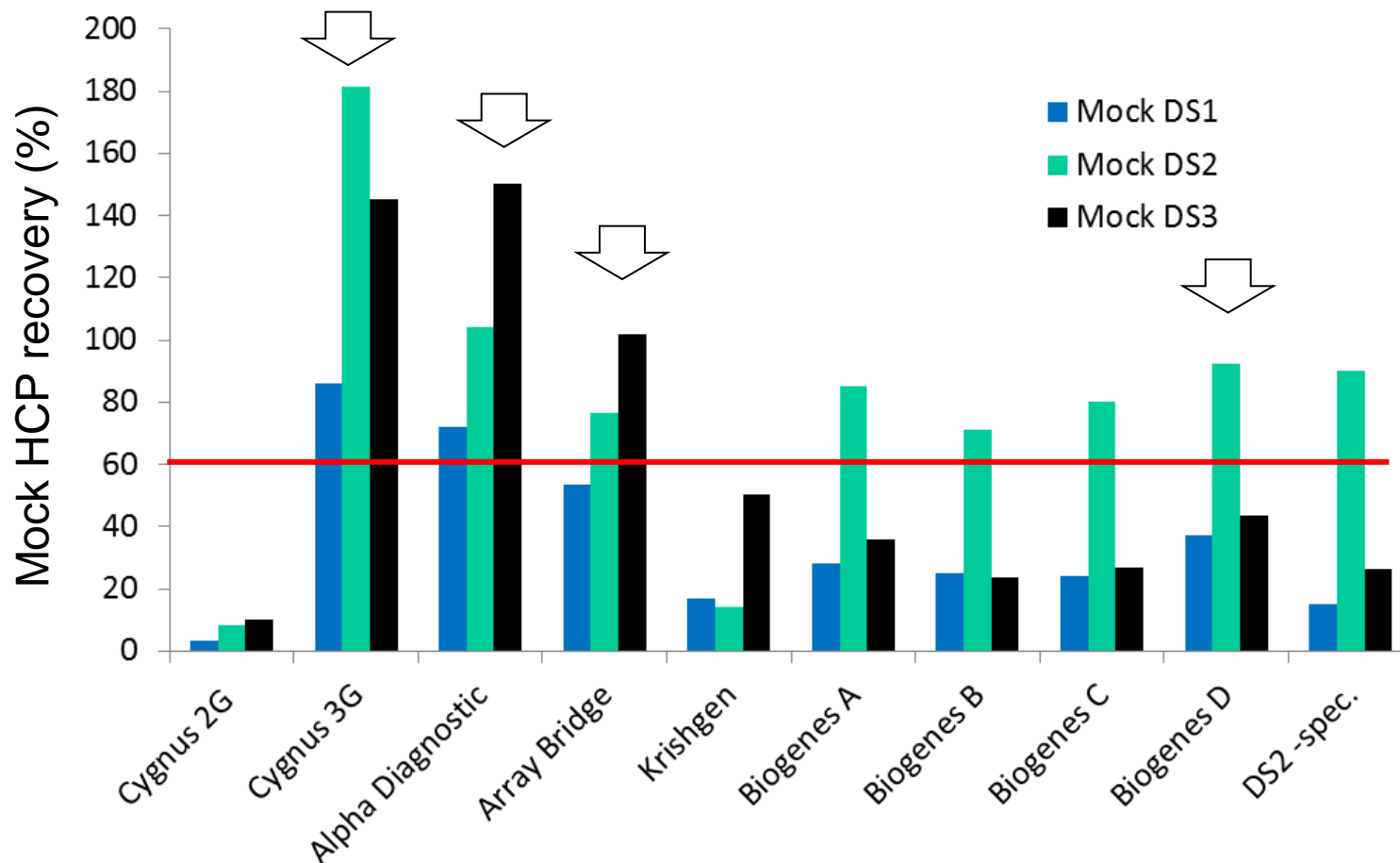
- Different recognition patterns
- Assays with high Mock HCP recovery lead to higher HCP values

Innovation, Science & Compliance
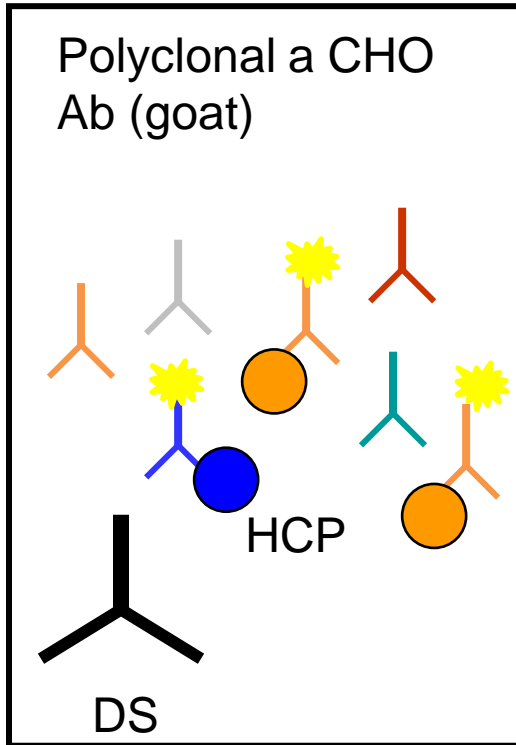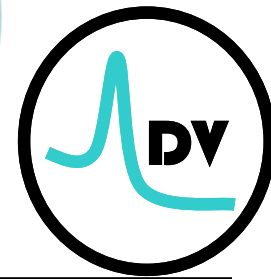
MSD
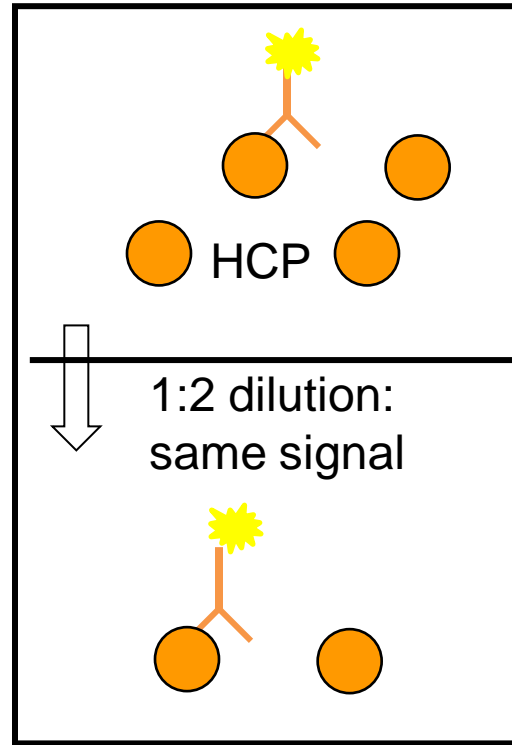*Be well*

Assessment of dilutional linearity

- Test 1 sample in different dilutions, assess HCP content in sample
- Dilutional linearity: similar values are generated for different dilutions (green)
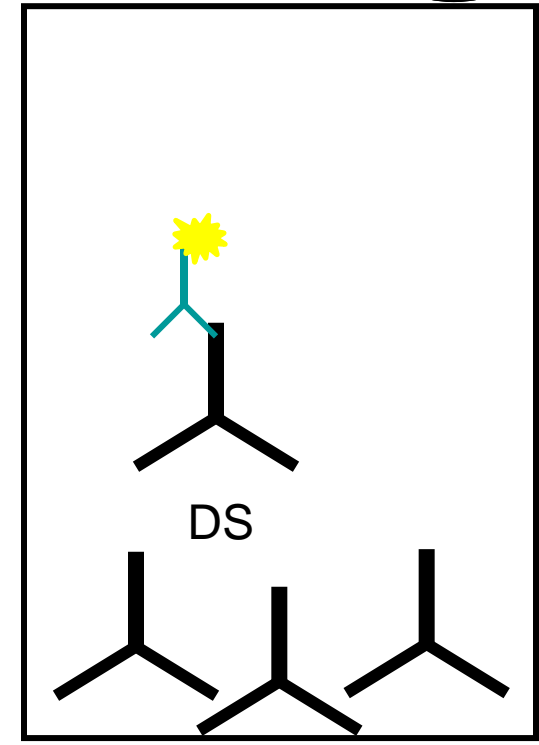- Prozone effect: duplication of values (red)
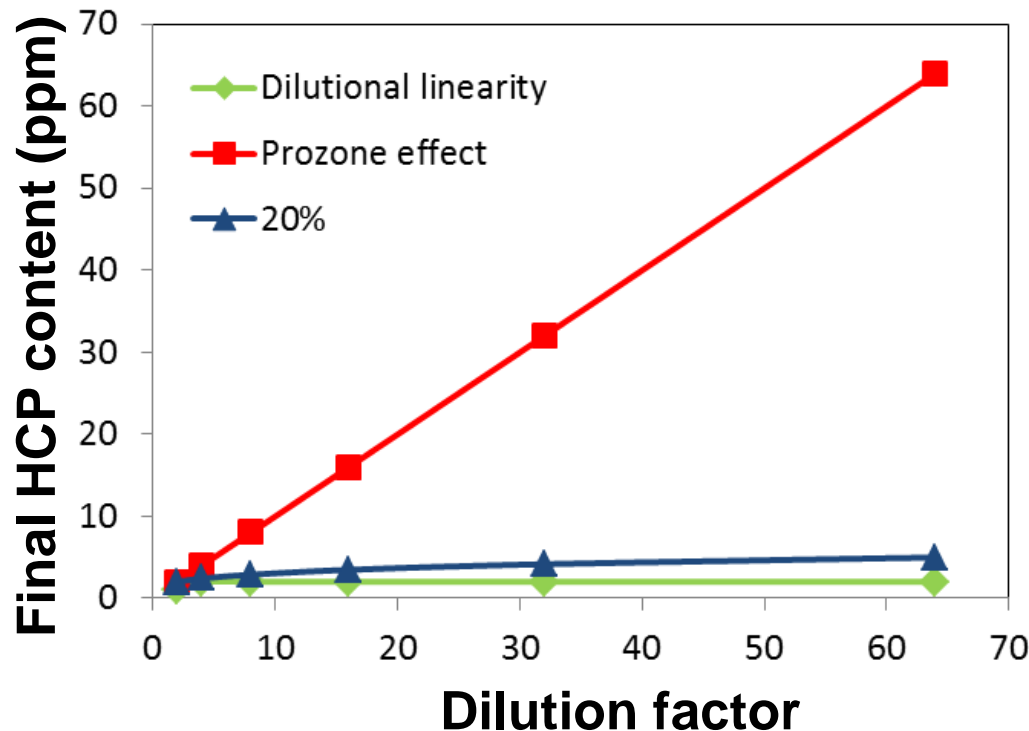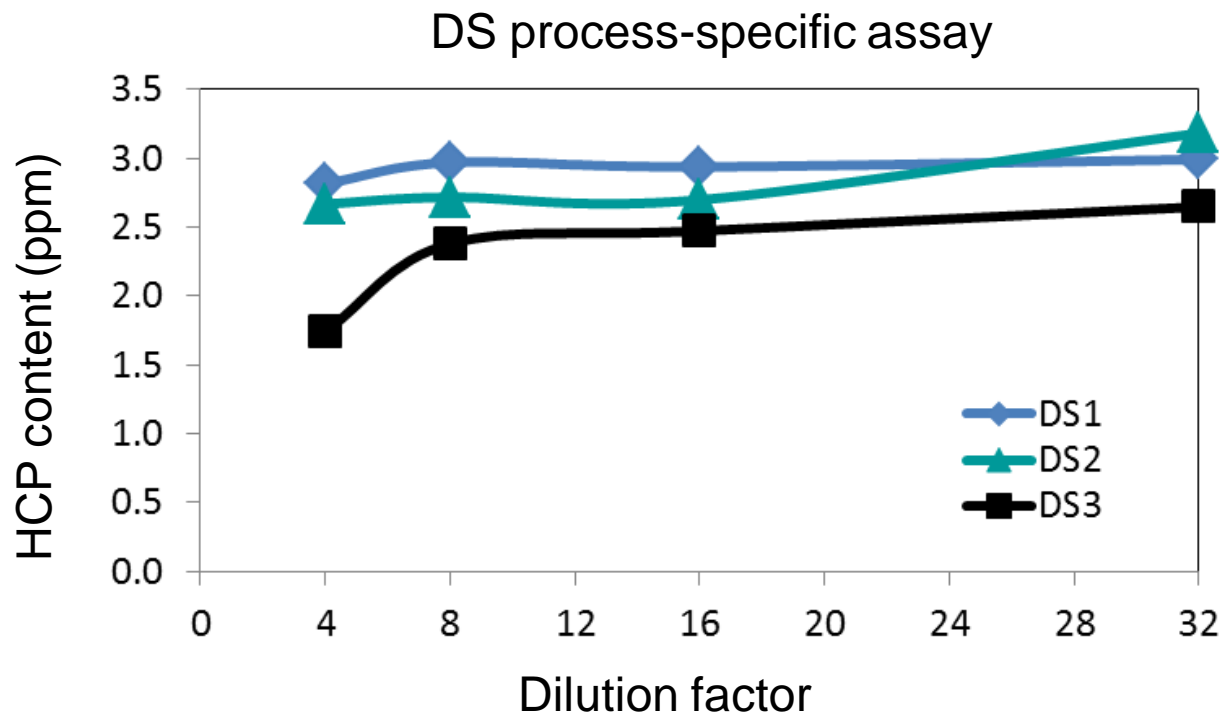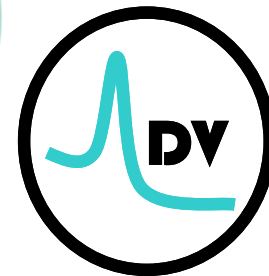
Possible explanations for lack of dilutional linearity

Ideal situation

Prozone effect: excess HCP

Cross-reactivity with DS

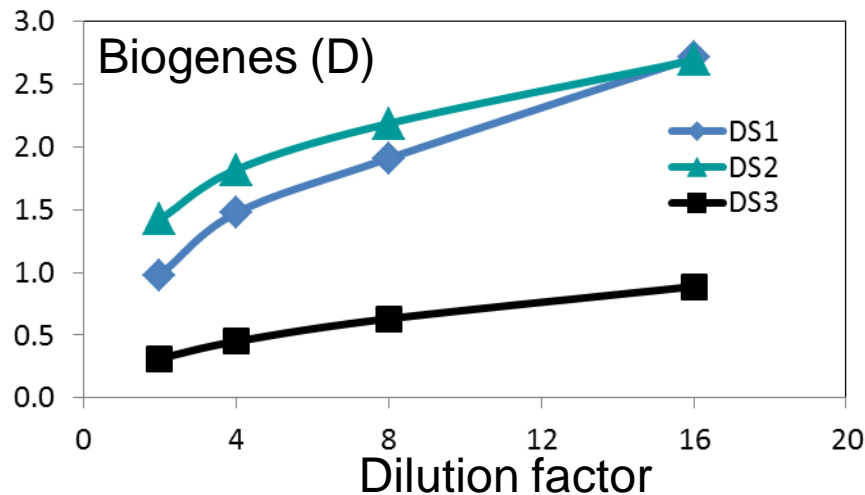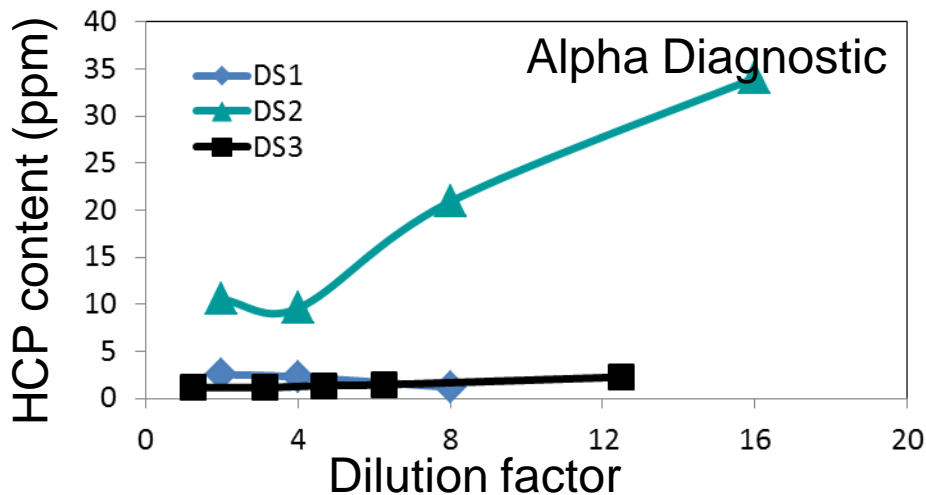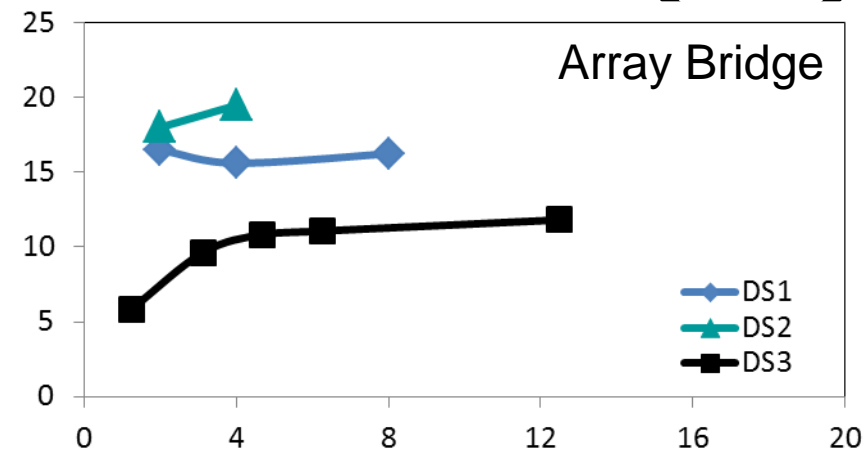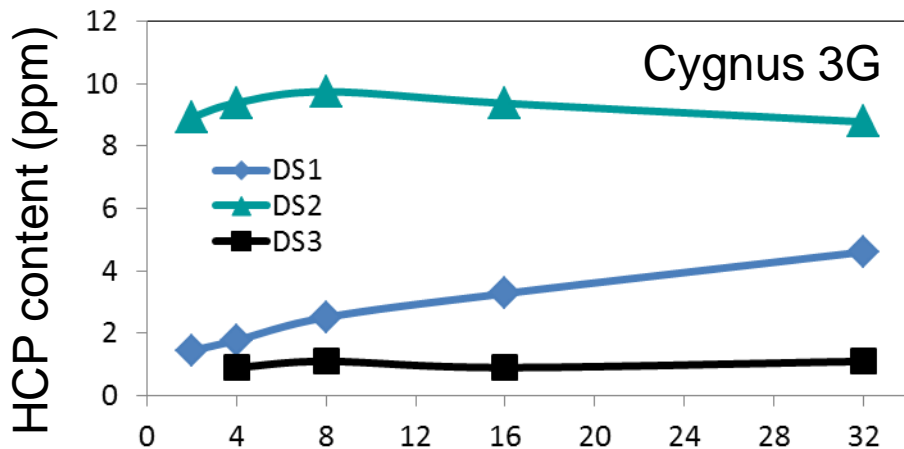- Lack of dilutional linearity (DL) often due to limited availability of Ab

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Assessment of dilutional linearity (DS2-specific assay)

DS process-specific assay

- DS process-specific assay displays dilutional linearity for all tested DS
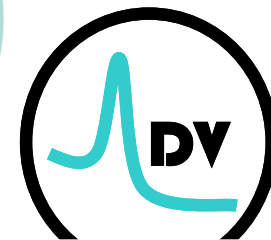
- Dilutional linearity is dependent on the combination of DS and kit

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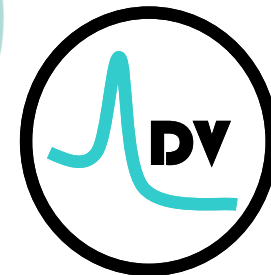
Conclusions commercial HCP ELISAs
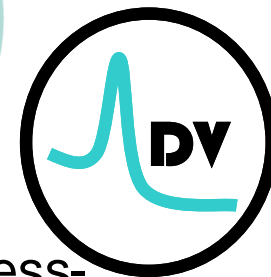
- Kits can be suitable tools for HCP detection until platform/process-specific assays are developed
  - Suitability should be assessed
  - Different kits seem to be suitable for different DS

	Mock HCP recovery above 60%	HCP content above QL	Dilutional linearity	Total score
Cygnus 2G	0	1	1	2
Cygnus 3G	3	3	2	**8**
Alpha Diagnostic	3	3	2	**8**
Array Bridge	2	3	2.5	**7.5**
Krishgen	0	1	1	2
Biogenes A	1	3	2	**6**
Biogenes B	1	3	1	5
Biogenes C	1	3	2	**6**
Biogenes D	1	3	1	5
DS2-spec	1	3	3	7

- 1 point per DS
- Max 3 points per criteria
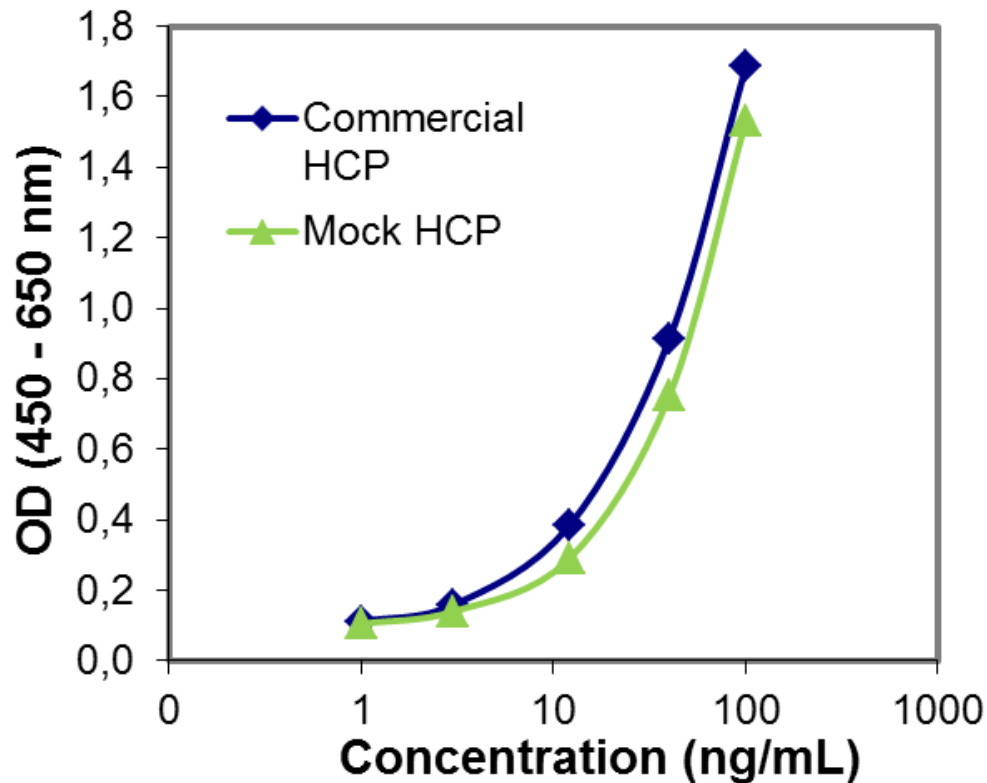
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Overview

- Introduction HCP, Detection by ELISA

- Comparison of commercially available kits

- Assessment of suitability of a commercial kit (Cygnus 3G kit for DS1)
  - Around 90% recovery of Mock DS1 HCP

- Conformational coverage determination

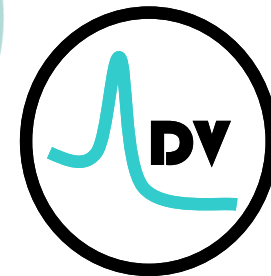# Comparison of Cygnus 3G and process-spec. HCP in Cygnus 3G kit
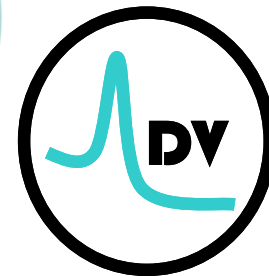
Commercial HCP ELISA (Cygnus 3G)

- DS Mock HCP look similar to commercial HCP in Cygnus 3G ELISA kit
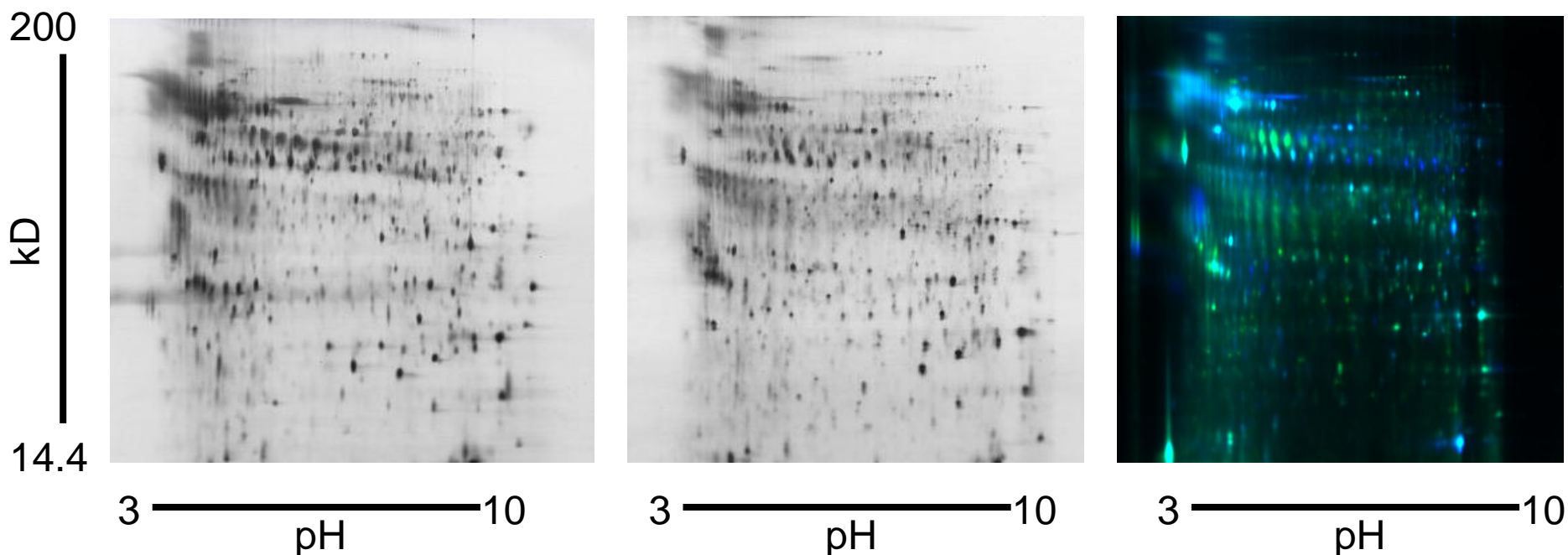
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Comparison 3G and process-spec. HCP
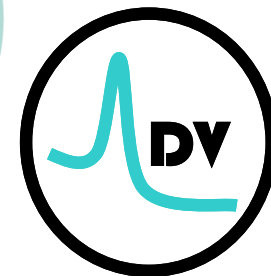
Mock HCP DS1
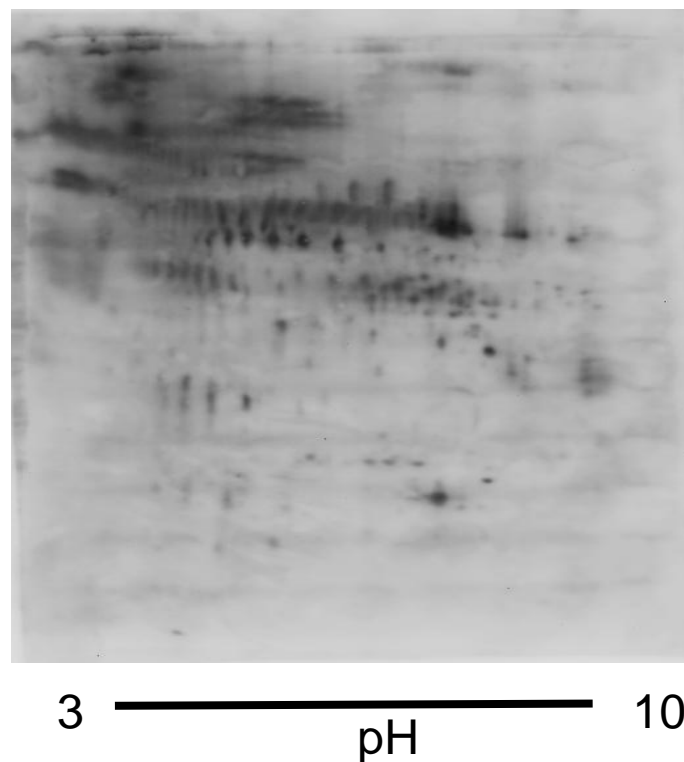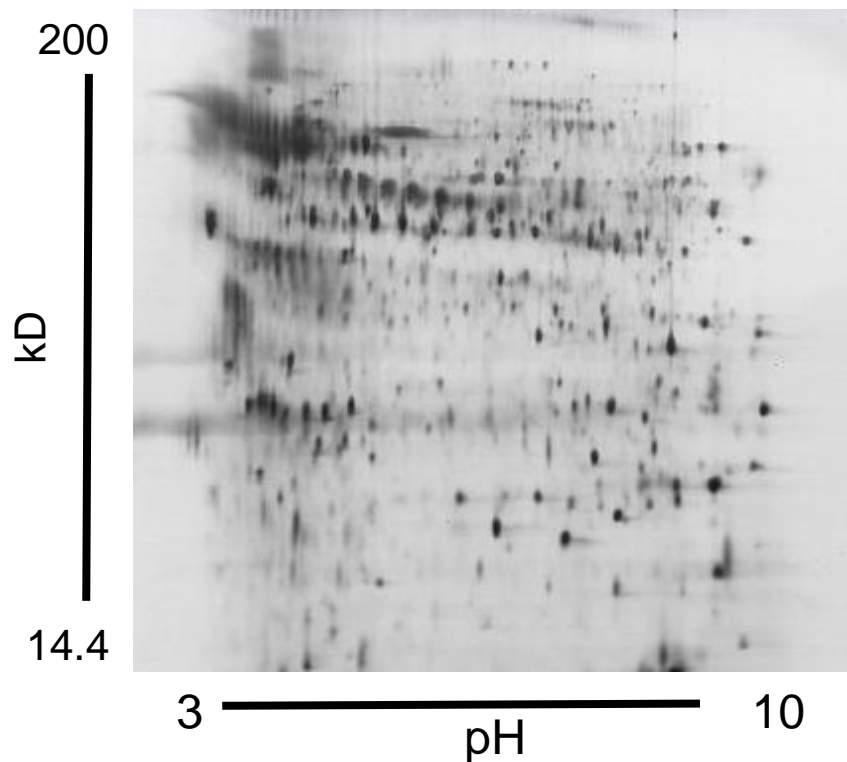Silver stain

Cygnus 3G HCP
Silver stain

DIGE Overlay
Fluorescence

- Differential gel electrophoresis (DIGE) quantitation: around 95% overlapping spots between process-spec. Mock HCP and commercial HCP

Innovation, Science & Compliance
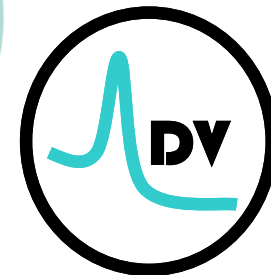
Coverage assessment 3G Ab

Mock HCP DS1
Silver stain

Mock HCP DS1
WB (Cygnus Ab)

- Coverage of commercial anti-HCP in 2D-GE: around 45%

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Overview

- Introduction HCP, Detection by ELISA

- Comparison of commercially available kits

- Assessment of suitability of a commercial kit

- Conformational coverage determination
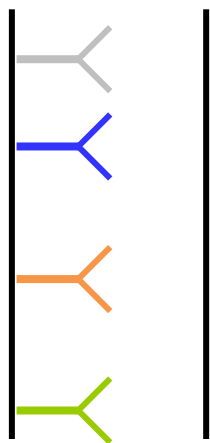
Coverage determination

- 2D gel electrophoresis is method of choice (silver stain, Western Blotting)

- Limitation: detection of linear epitopes

- Assess coverage in dilution

- Depletion assay to assess conformational coverage
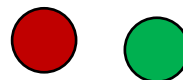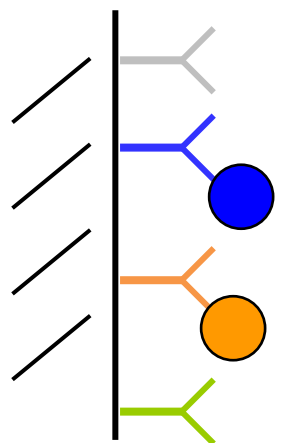
Innovation, Science & Compliance

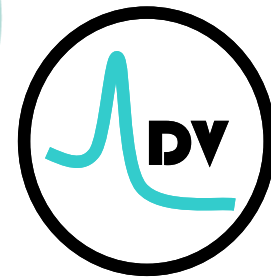# Outline depletion assay
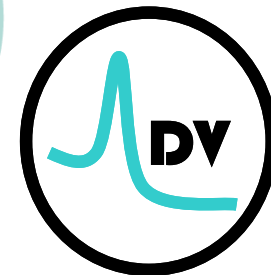
Couple pAb to
Prot G/NHS sepharose

Apply Mock CHO proteins
Proteins recognised by pAB will bind

Collect flow-through
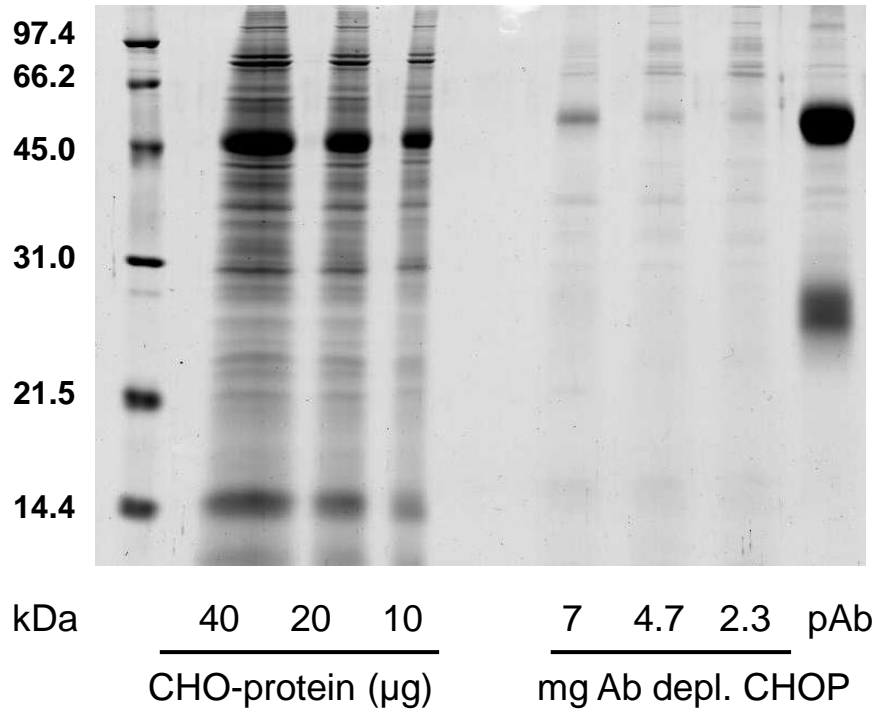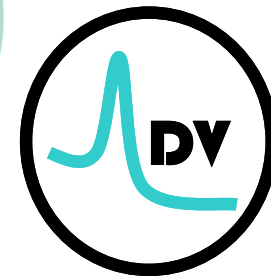Analyze with
SDS-PAGE or LC-MS

SDS-PAGE, Gelcode Blue Stain

Example: Coverage determination by depletion assay

SDS-PAGE, Gelcode Blue Stain

$$y = 4.9489x - 0.9954$$
$$R^2 = 0.9999$$

Adj. Volume (ODxmm^2) vs Amount CHO proteins (ug)
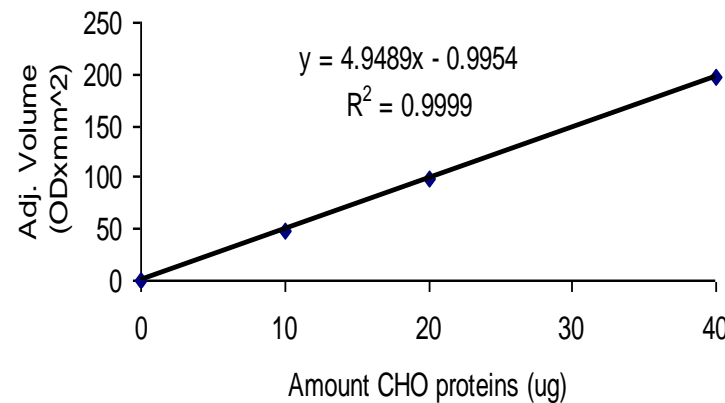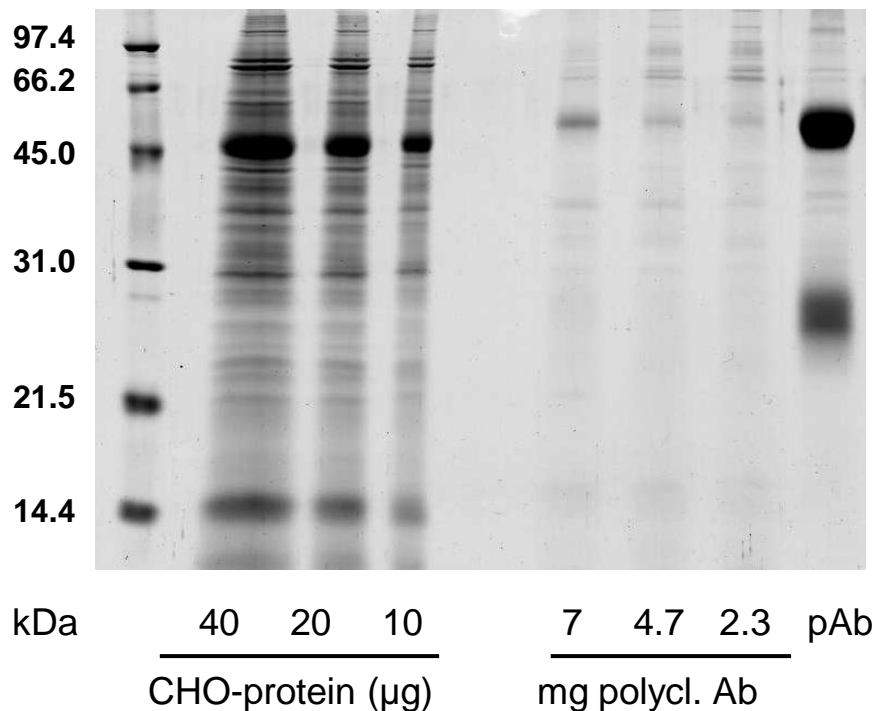
aCHO IgG (mg)	CHOP (mg)	Adj Volume (ODxmm^2)	Recovery (ug)	Recovery (%)
2.33	0.1	5.95	1.4	2.8
4.66	0.1	6.4	1.5	3.0
7.00	0.1	7.2	1.7	3.3

Gel lane labels:

kDa: 97.4, 66.2, 45.0, 31.0, 21.5, 14.4

CHO-protein (µg): 40 20 10

mg polycl. Ab: 7 4.7 2.3 pAb

- Around 97% coverage of rabbit pAb for conformational epitopes

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MSD — Be well

Summary and conclusions
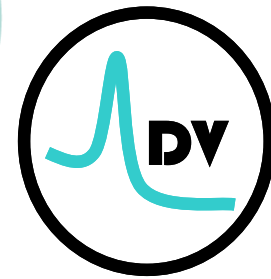
- Kits can be suitable tools for HCP detection until platform/process-specific assays are developed
  - Suitability should be assessed
    - Comparison of HCP
    - Dilutional linearity
    - Coverage assessment recommended

- Lack of dilutional linearity should be further investigated and might be due to cross-reactivity with DS or scarcity of individual Ab

- Recommendation to harmonize coverage determination by 2D-GE and expand coverage assessment to methods detecting conformational coverage

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Acknowledgements
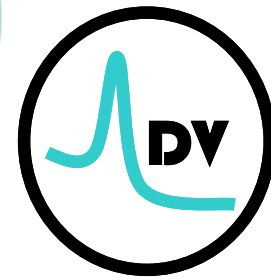
Analytical Development & Validation, MSD Oss, NL

- Corné Stroop
- Bert v. d. Weijer
- Danny Lagarde
- Loes Schobers
- Rezie te Poele
- Rik Nievergeld
- Sjuul Hegger

Process Development & Commercialization, MSD Oss, NL

- Rick Schreurs
- Manon Bruisten
- Nora Renders

Extended Characterization BioChemical, MSD Oss, NL

- Eef Dirksen
- Wendy Pluk
- Wilbert Deceunink

Extended Characterization, Merck Kenilworth, USA

- Alex Ambrogelli
- Dennis Driscoll
- Shara Dellatore
- Daisy Richardson

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