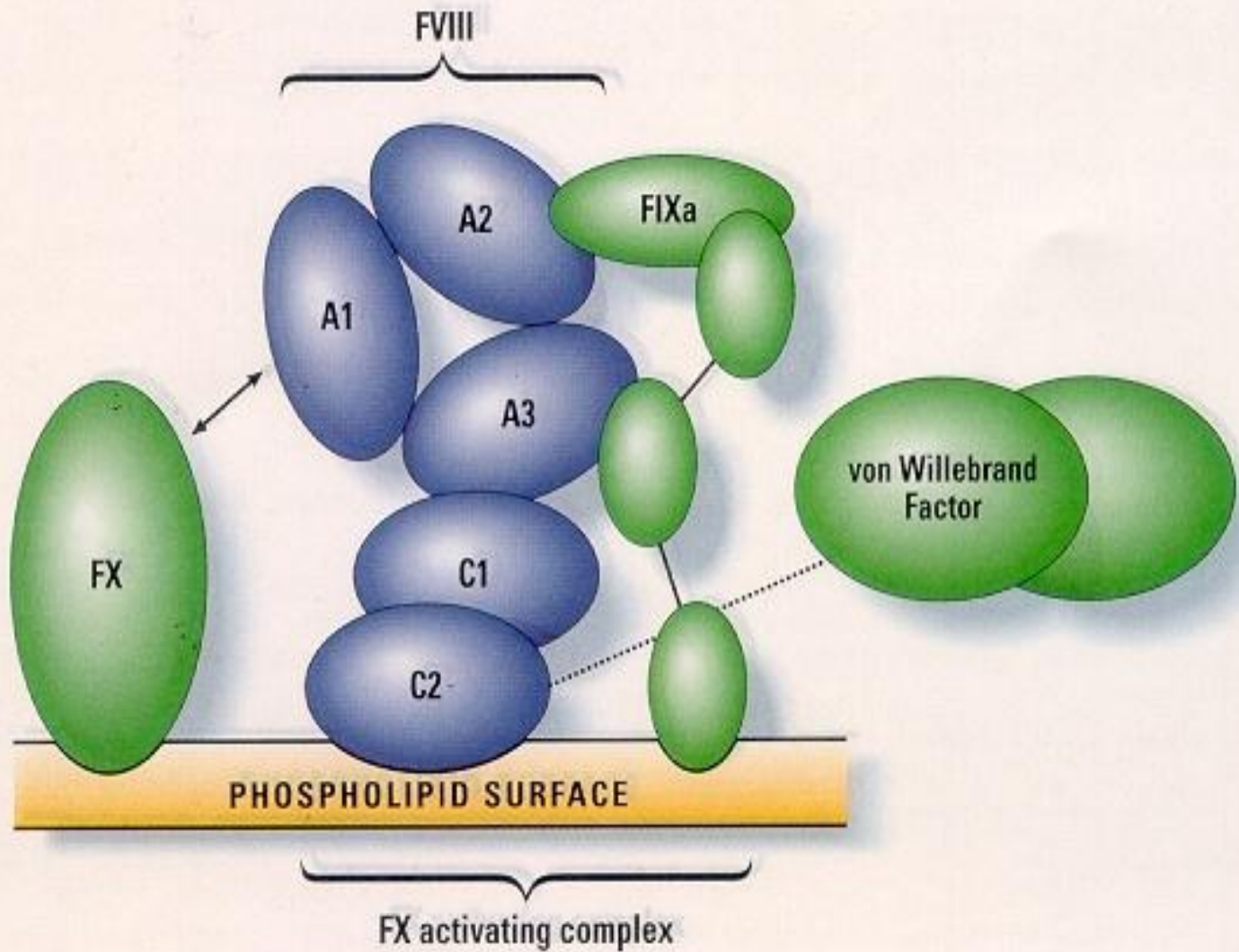
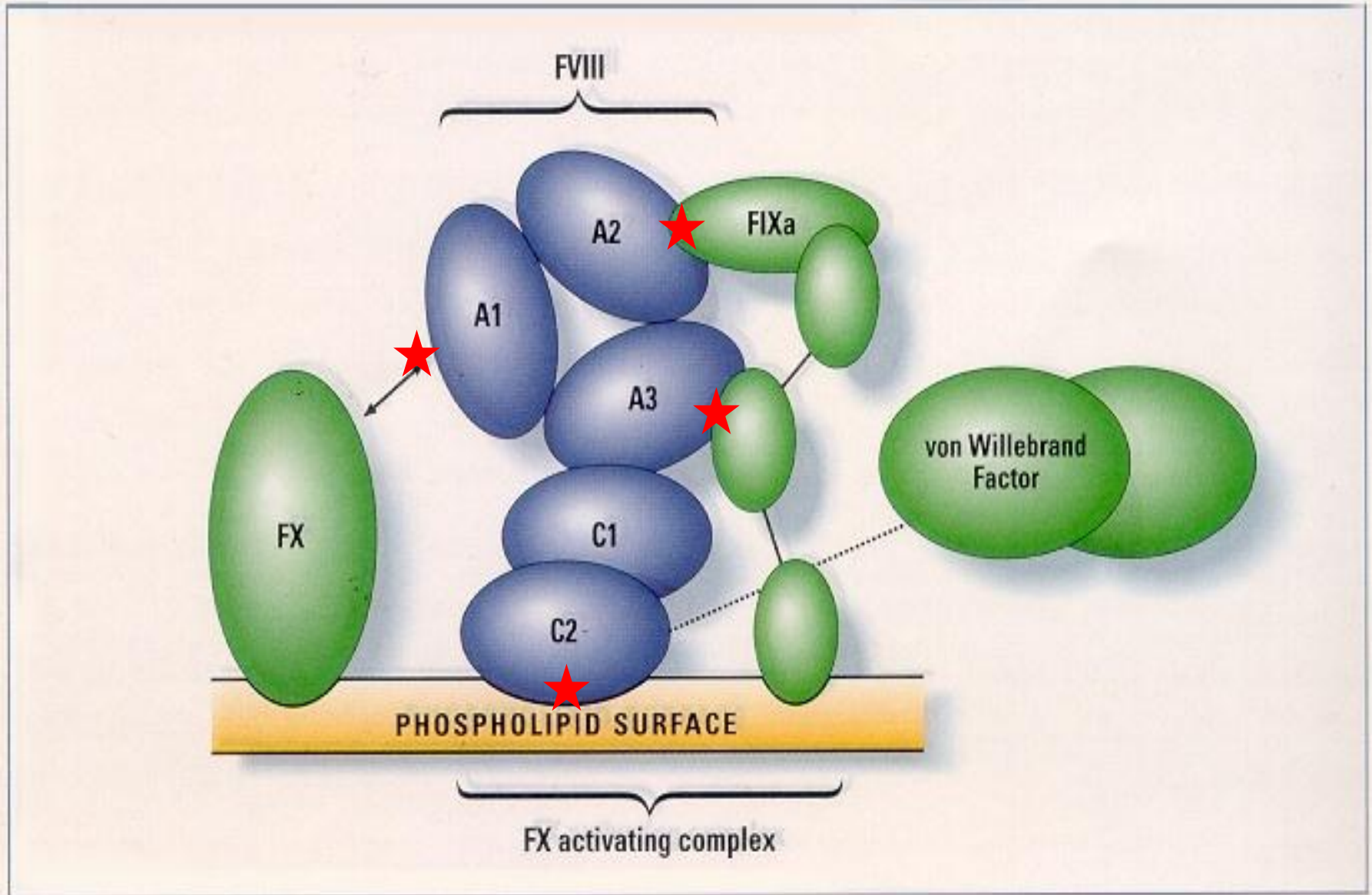


Factor VIII Inhibitor Testing: The Way to Better Comparison of Results

Bert Verbruggen



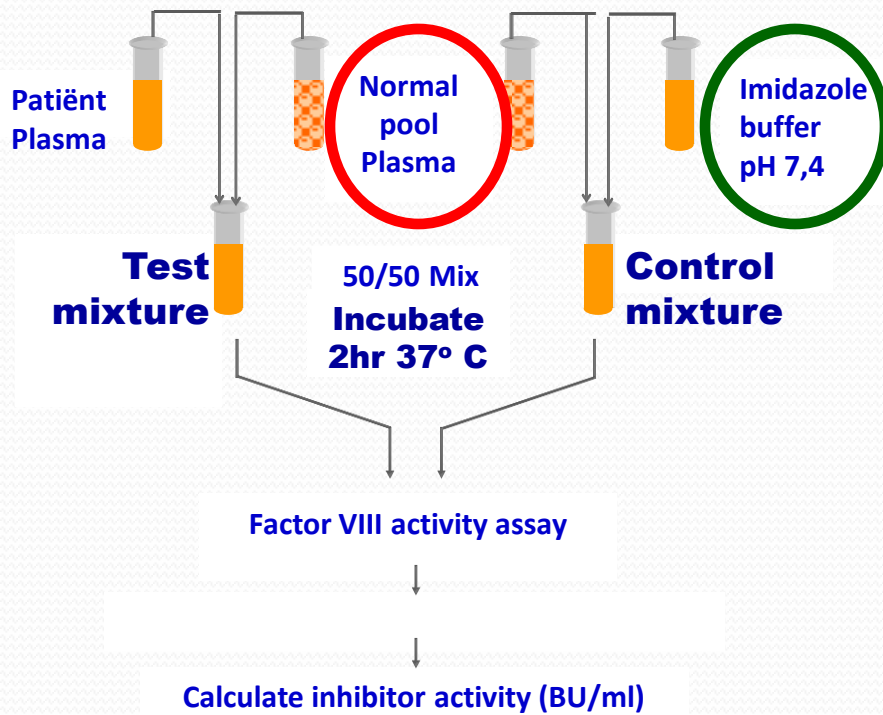


Introduction Factor VIII Inhibitors

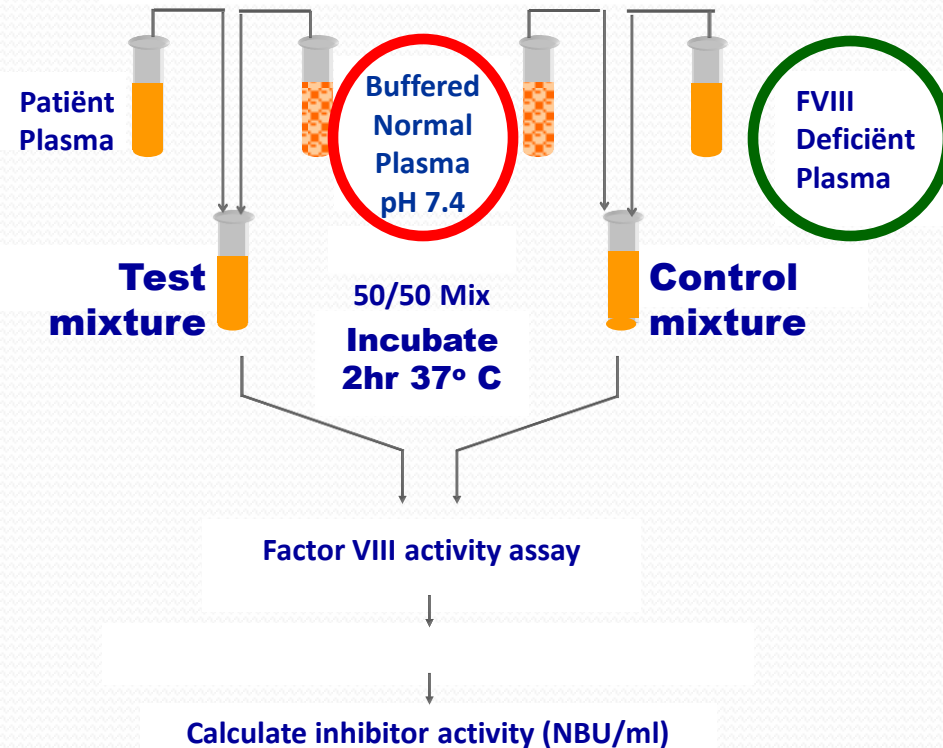
- **In up to 45% of severe haemophilia patients, Factor VIII inhibitors occur as a result of treatment with Factor VIII products.**
- **Reliable and reproducible inhibitor testing is important for early detection in initial phase, monitoring during treatment and confirmation of eradication at the end of therapy.**
- **Standardization and external surveys are essential steps in quality management**

Methodology Factor VIII Inhibitor Assay

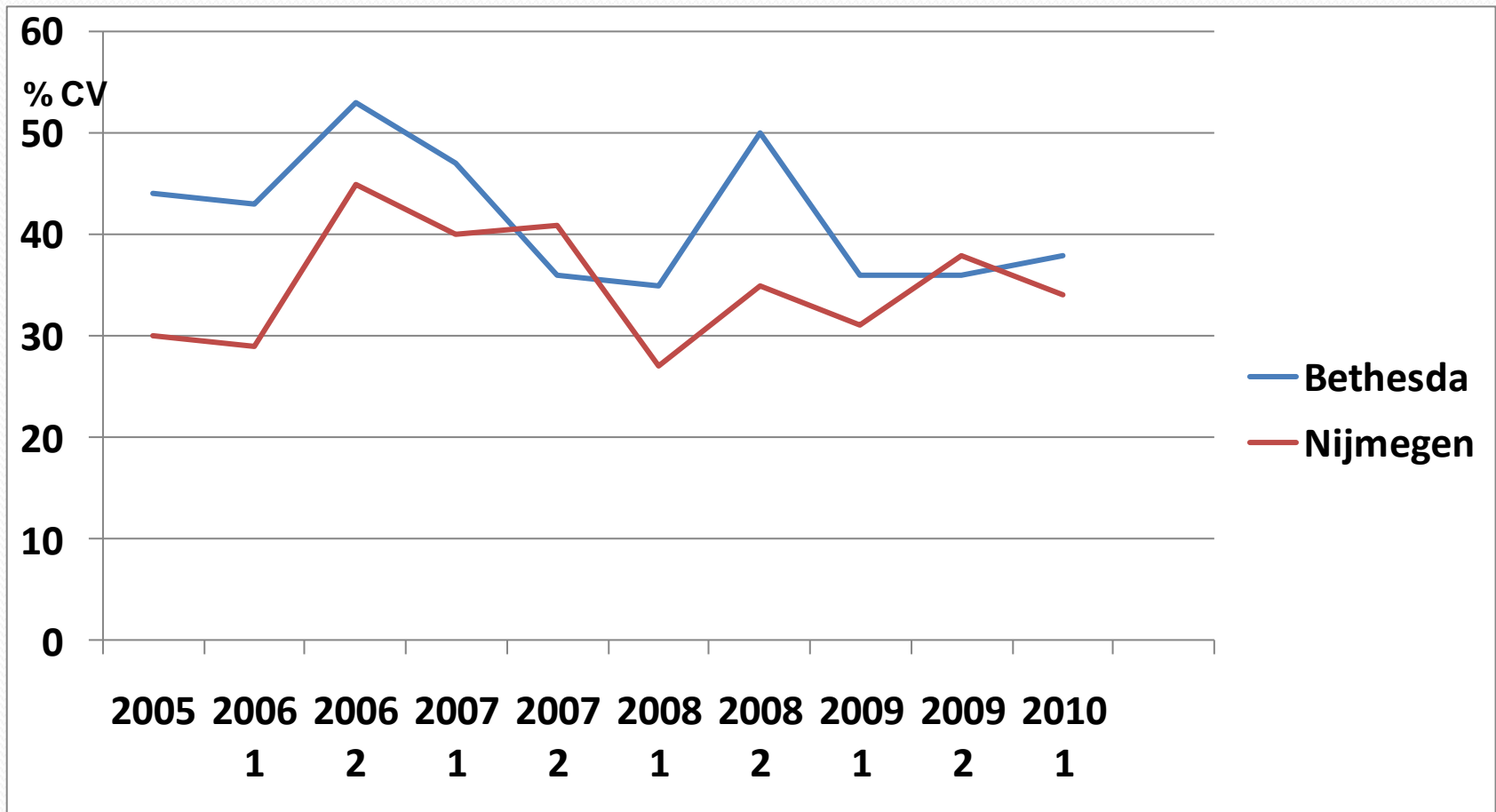
BETHESDA ASSAY



NIJMEGEN ASSAY



Coefficient of Variation of ECAT Surveys since 2005



<u>ECAT Surveys</u> <u>Factor VIII inhibitor (BU/mL)</u>	n	Mean (range)	Number falsely pos	Outliers	Number falsely negative	CV (%) Bethesda Nijmegen
Sample A 2005	132	14.9 (2 – 67)	-	4	0	41 / 28
Sample B 2005	131	1.9 (0 – 42)	-	7	11	48 / 32
Sample C 2005 (neg)	127	- (0 – 8)	7	-	-	-
Sample 2006.31	104	1.3 (0.3 – 3.6)	-	2	0	43 / 29
Sample 2006.32 (neg)	102	- (0 – 6.4)	8	-	-	-
Sample 2006.64	107	1.7 (0.4 – 16)	-	1	7	53 / 45
Sample 2006.63 (neg)	109	- (0 – 2.2)	18	-	-	-
Sample 2007.31	124	1.0 (0.0 – 2.4)	-	2	9	47 / 40
Sample 2007.32 (neg)	126	0.5 (0.0 – 2.0)	?	2	-	-
Sample 2007.65	148	1.4 (0.0 – 3.0)	-	3	4	36 / 38
Sample 2007.66	146	3.1 (0 – 7.6)	-	3	?	36 / 45
Sample 2008.33	172	3.0 (1.1 – 6.0)	-	3	?	29 / 25
Sample 2008.34	172	1.3 (0.3 – 3.1)	-	1	5	41 / 29
Sample 2008.74 (neg)	164	0.3 (0.1 – 2.5)	8	3	-	-
Sample 2008.75	164	0.9 (0.2 – 13.5)	-	1	21	50 / 35
Sample 2009.35	152	5.6 (0 – 45)	-	3	1	32 / 32
Sample 2009.36	143	1.0 (0 – 147)	-	4	5	40 / 29
Sample 2009.80	153	3.4 (0.0 – 16.0)	-	1	2	36 / 38
Sample 2009.81 (neg)	152	0.4 (0.1 – 1.5)	48	-	-	-

<u>ECAT Surveys</u> <u>Factor VIII inhibitor (BU/mL)</u>	n	Mean (range)	Number falsely pos	Outliers	Number falsely negative	CV (%) Bethesda Nijmegen
Sample A 2005	132	14.9 (2 – 67)	-	4	0	41 / 28
Sample B 2005	131	1.9 (0 – 42)	-	7	11	48 / 32
Sample C 2005 (neg)	127	- (0 – 8)	7	-	-	-
Sample 2006.31	104	1.3 (0.3 – 3.6)	-	2	0	43 / 29
Sample 2006.32 (neg)	102	- (0 – 6.4)	8	-	-	-
Sample 2006.64	107	1.7 (0.4 – 16)	-	1	7	53 / 45
Sample 2006.63 (neg)	109	- (0 – 2.2)	18	-	-	-
Sample 2007.31	124	1.0 (0.0 – 2.4)	-	2	9	47 / 40
Sample 2007.32 (neg)	126	0.5 (0.0 – 2.0)	?	2	-	-
Sample 2007.65	148	1.4 (0.0 – 3.0)	-	3	4	36 / 38
Sample 2007.66	146	3.1 (0 – 7.6)	-	3	?	36 / 45
Sample 2008.33	172	3.0 (1.1 – 6.0)	-	3	?	29 / 25
Sample 2008.34	172	1.3 (0.3 – 3.1)	-	1	5	41 / 29
Sample 2008.74 (neg)	164	0.3 (0.1 – 2.5)	8	3	-	-
Sample 2008.75	164	0.9 (0.2 – 13.5)	-	1	21	50 / 35
Sample 2009.35	152	5.6 (0 – 45)	-	3	1	32 / 32
Sample 2009.36	143	1.0 (0 – 147)	-	4	5	40 / 29
Sample 2009.80	153	3.4 (0.0 – 16.0)	-	1	2	36 / 38
Sample 2009.81 (neg)	152	0.4 (0.1 – 1.5)	48	-	-	-

F VIII Inhibitor Testing: The Way to better Comparison of Results!!

F VIII Inhibitor Testing: The Way to better Comparison of Results!!

Which way ??

F VIII Inhibitor Testing: The Way to better Comparison of Results!!

Which way ??



F VIII Inhibitor Testing: The Way to better Comparison of Results!!

Which way ??





FVIII inhibitor Workshop November 2009



Aim of the workshop

The aim of the workshop was to investigate components that contribute to the high inter-laboratory variability of the results of the factor VIII inhibitor assays (observed in ECAT surveys) and to come up with suggestions to reduce the variability of the assay.

Design of the workshop

- **Pre-workshop survey (7 samples) in 51 laboratories (June 2009)**
- **16 Participants from 9 different countries using different methodologies and showing a wide range of inter-laboratory variation were invited for the workshop**
- **During the workshop 4 runs over three days**
- **Variation in assay conditions**

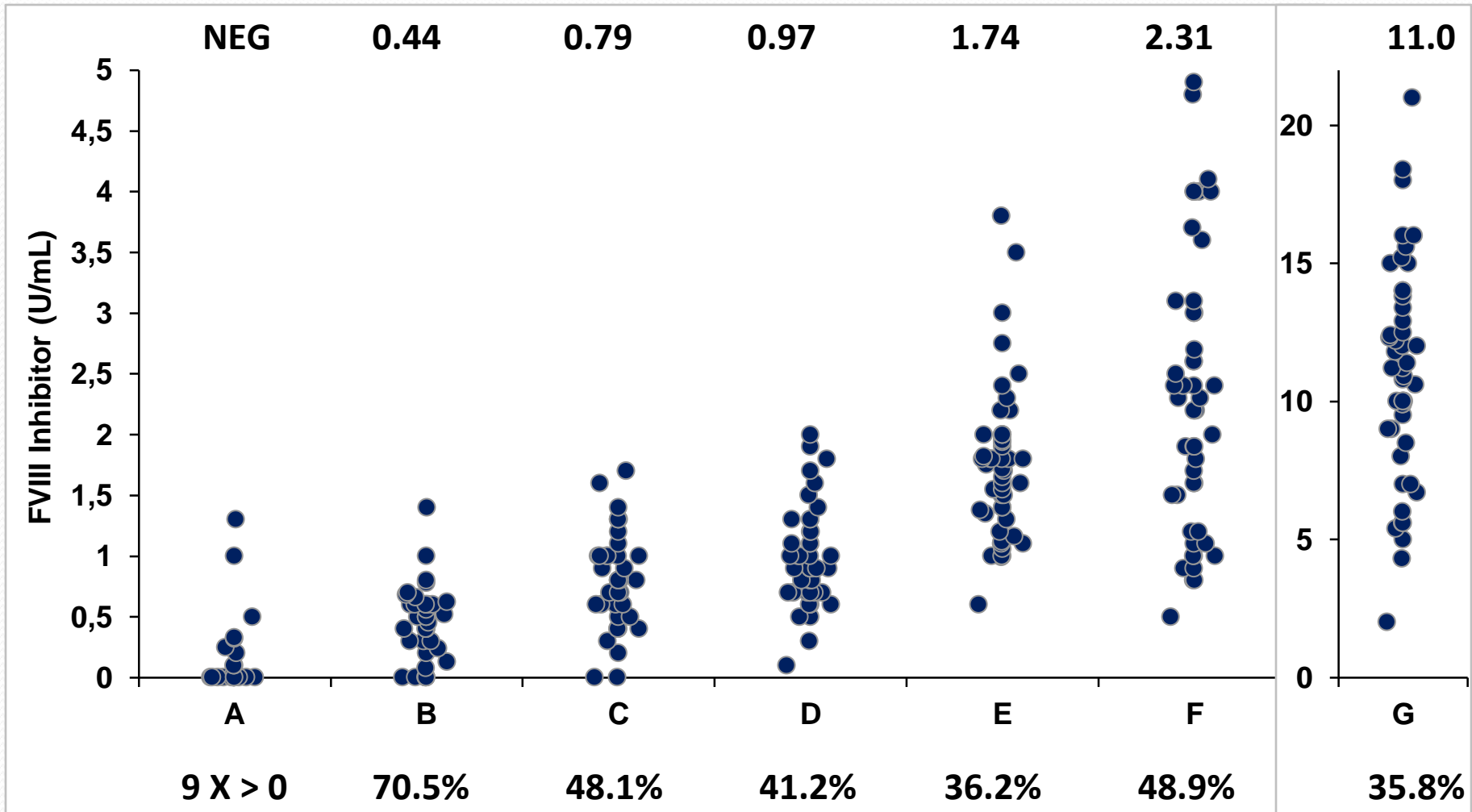
Sessions

SESSION	DESCRIPTION
0	Pre-workshop survey / home method
1	Repeat home method on central location, with reagents and dilutions as used in survey
2	Buffered (own) NP / FVIII def plasma / dilutions as used in survey / own reagents for FVIII measurement
3	Buffered universal NP / universal FVIII def / universal dilutions / own FVIII deficient plasma and reagents for FVIII measurement
4	Nijmegen assay with universal reagents / universal dilutions / universal reagents for FVIII measurement

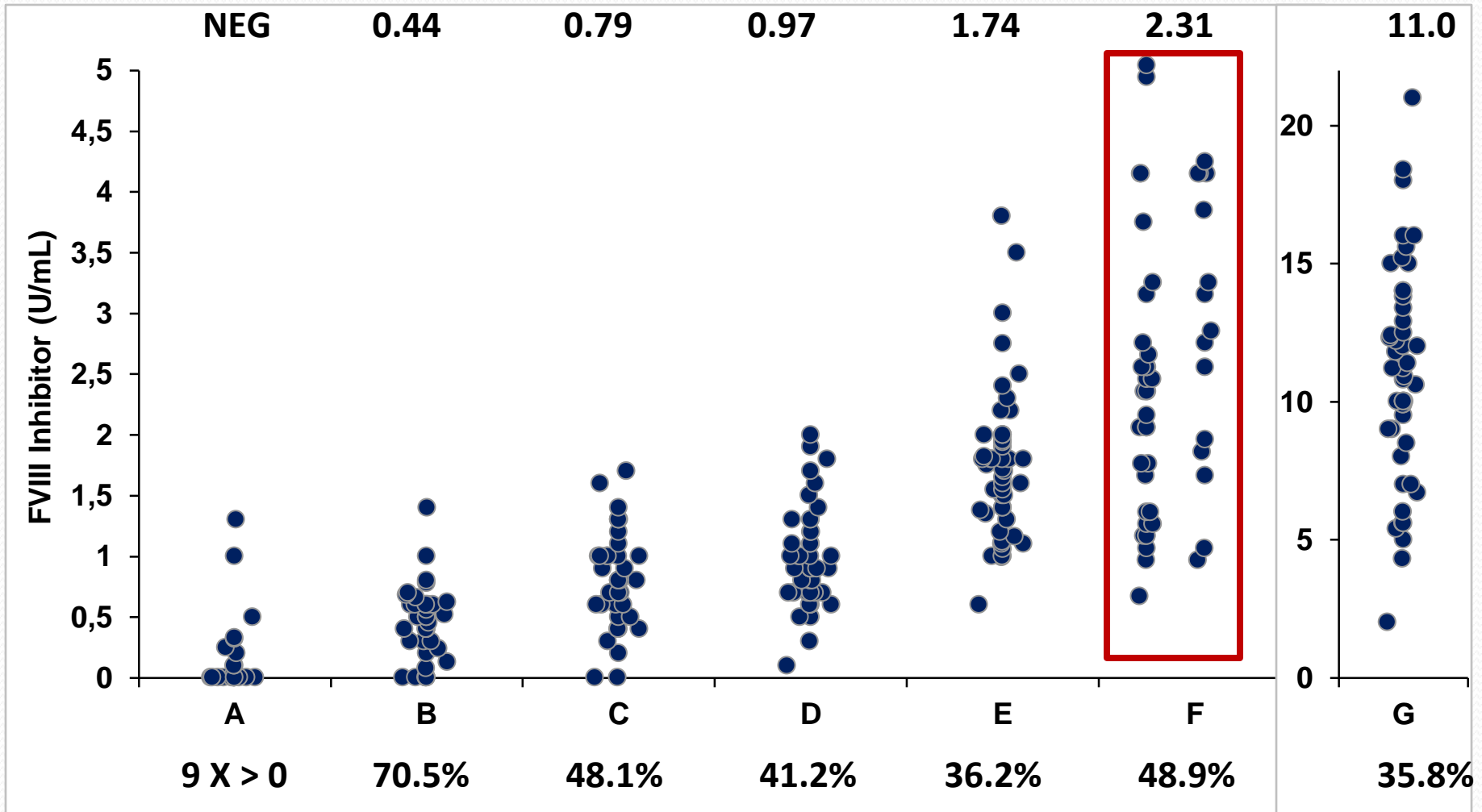
Samples

SAMPLE	BU/mL	DESCRIPTION
1	1.60	Monoclonal Ab against C2-domain
2	0.81	Monoclonal Ab against A1-domain
3	1.40	Moderate titre patient sample
4	0.74	Low titre 1:1 dilution of sample 3
5	1.94	Moderate titre patient sample
6	15.4	High titre polyclonal inhibitor
7	-	Negative control

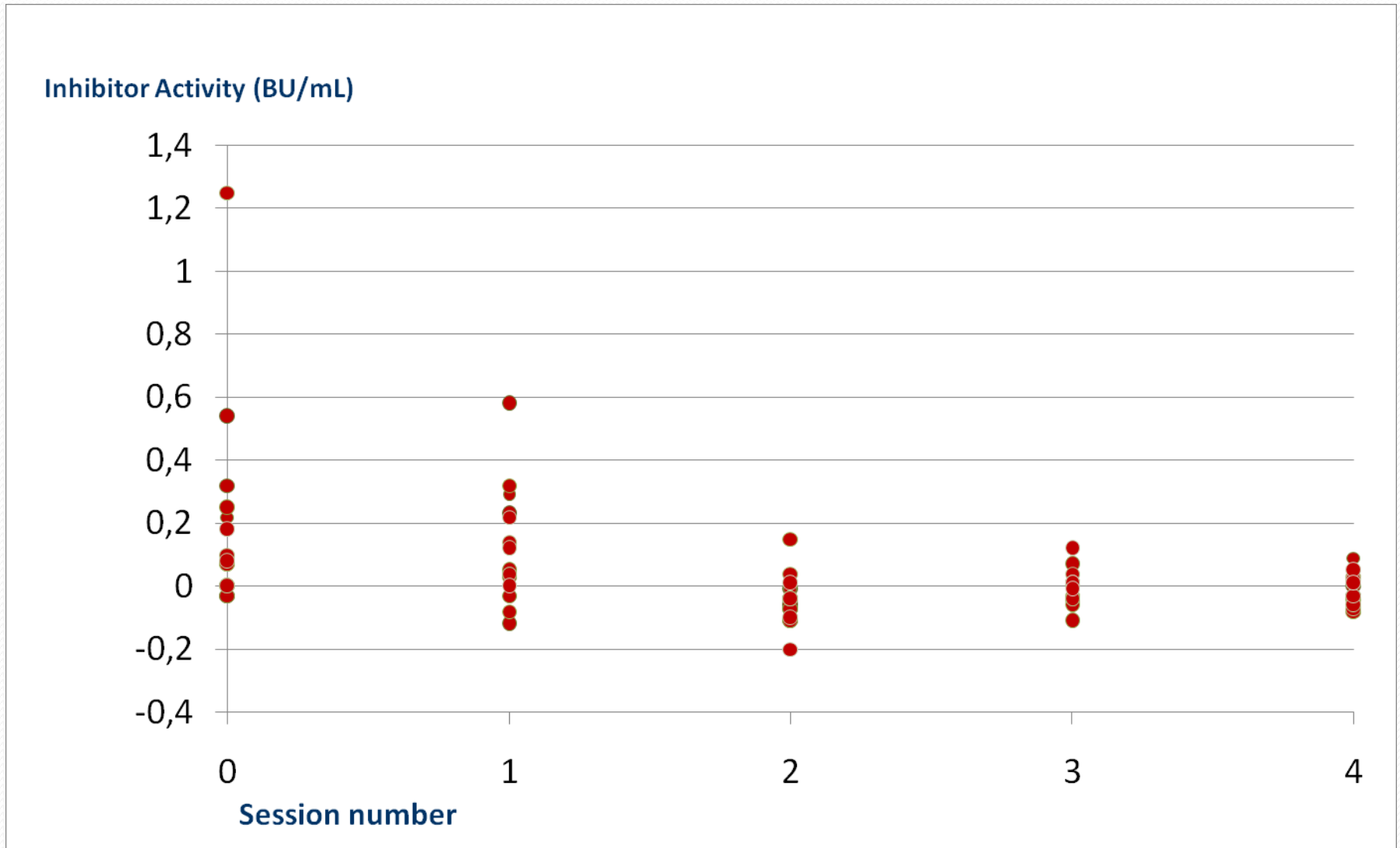
Pre-Workshop Survey



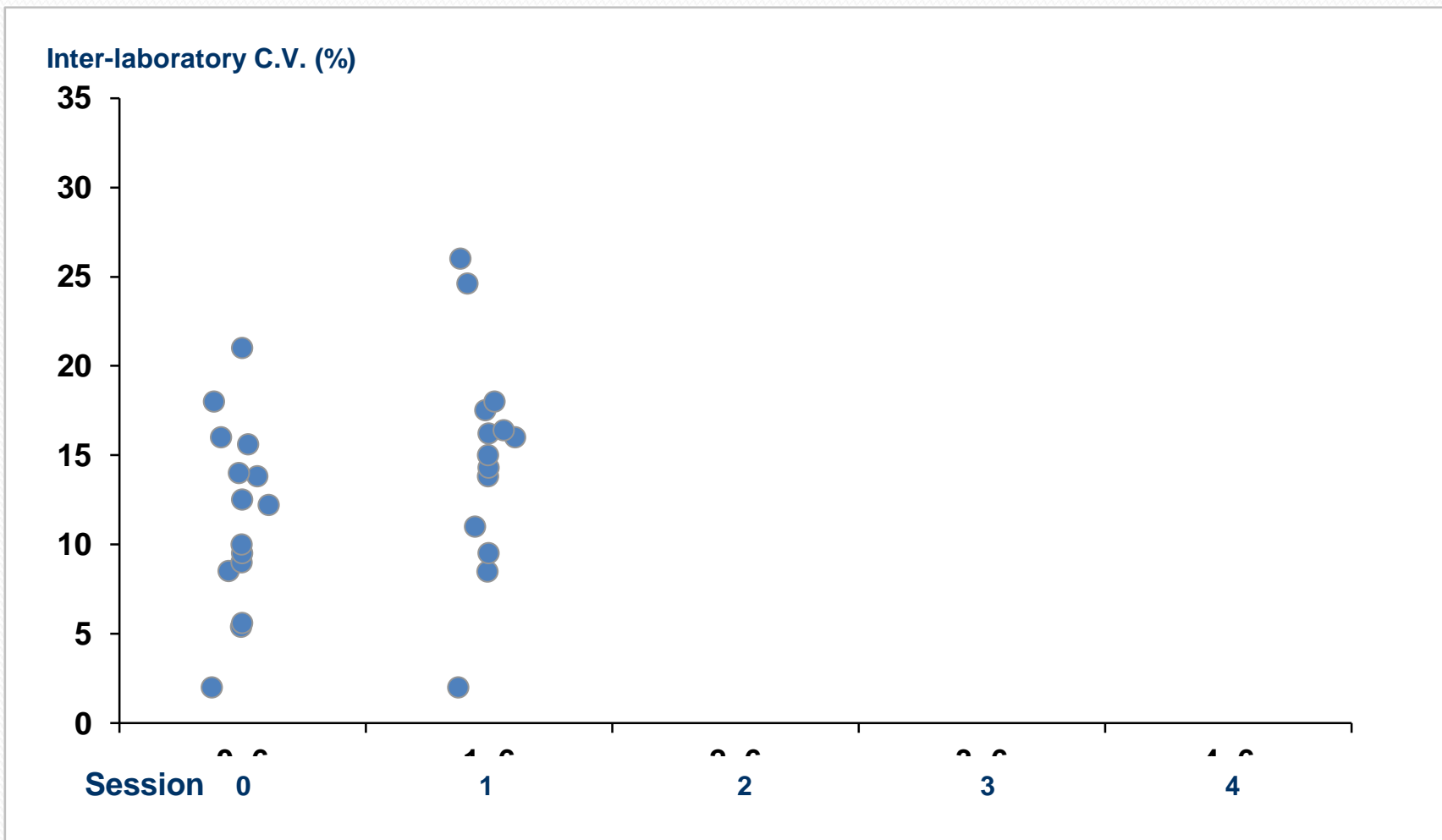
Pre-Workshop Survey



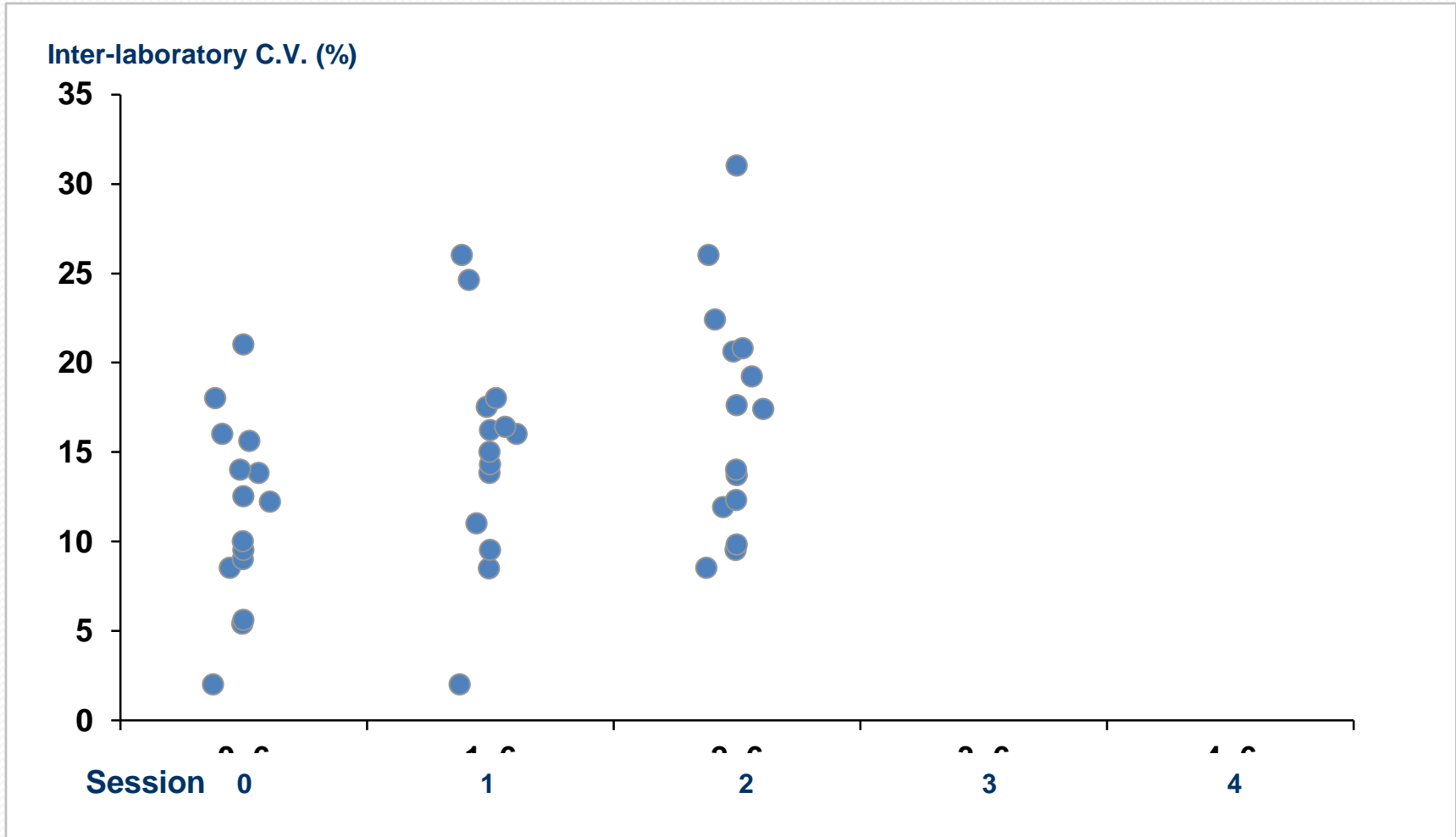
FVIII Inhibitor Activity of Inhibitor Negative Sample in Different Sessions



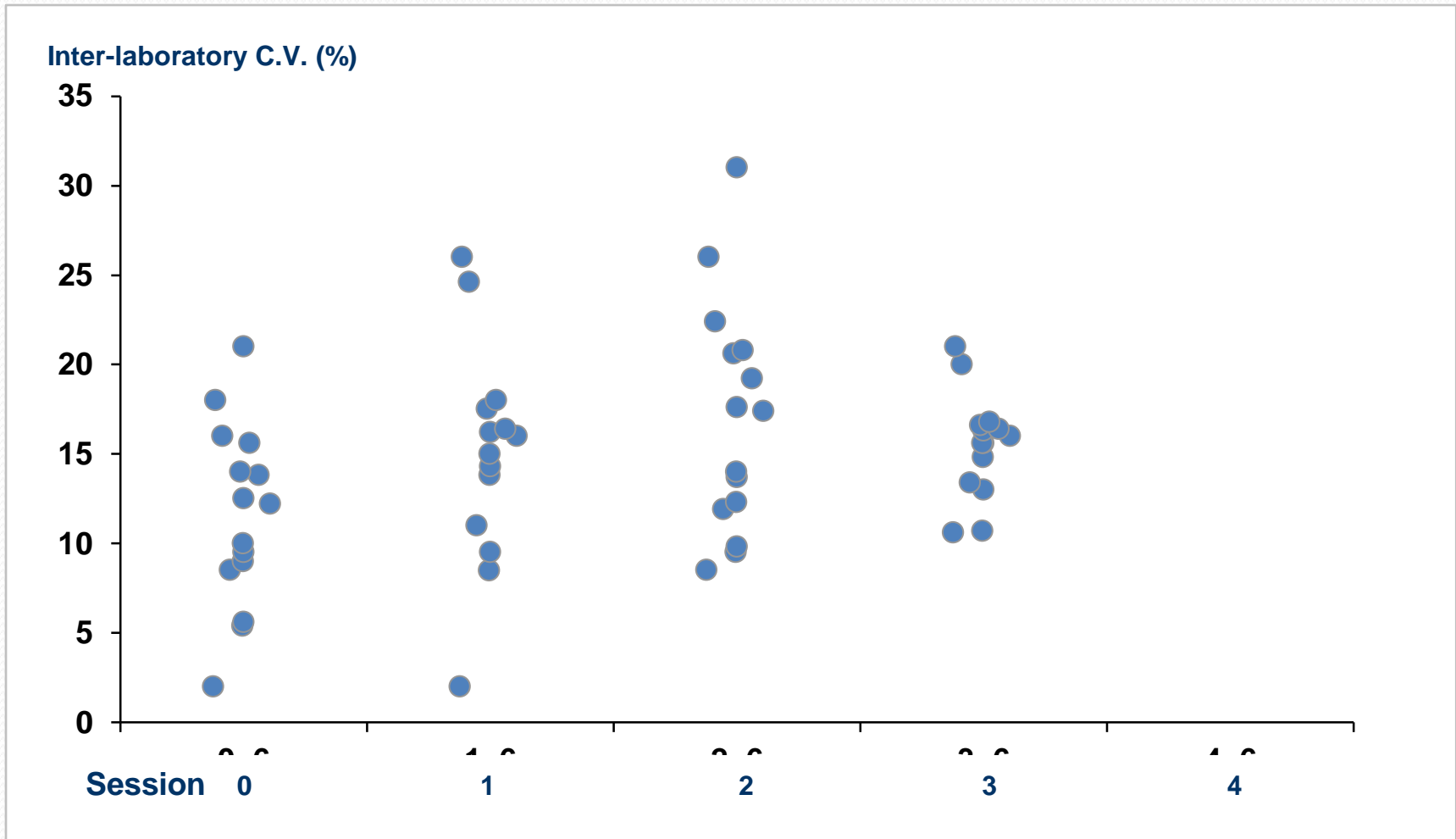
Inter-laboratory C.V. of Sample 6 in Consecutive Sessions



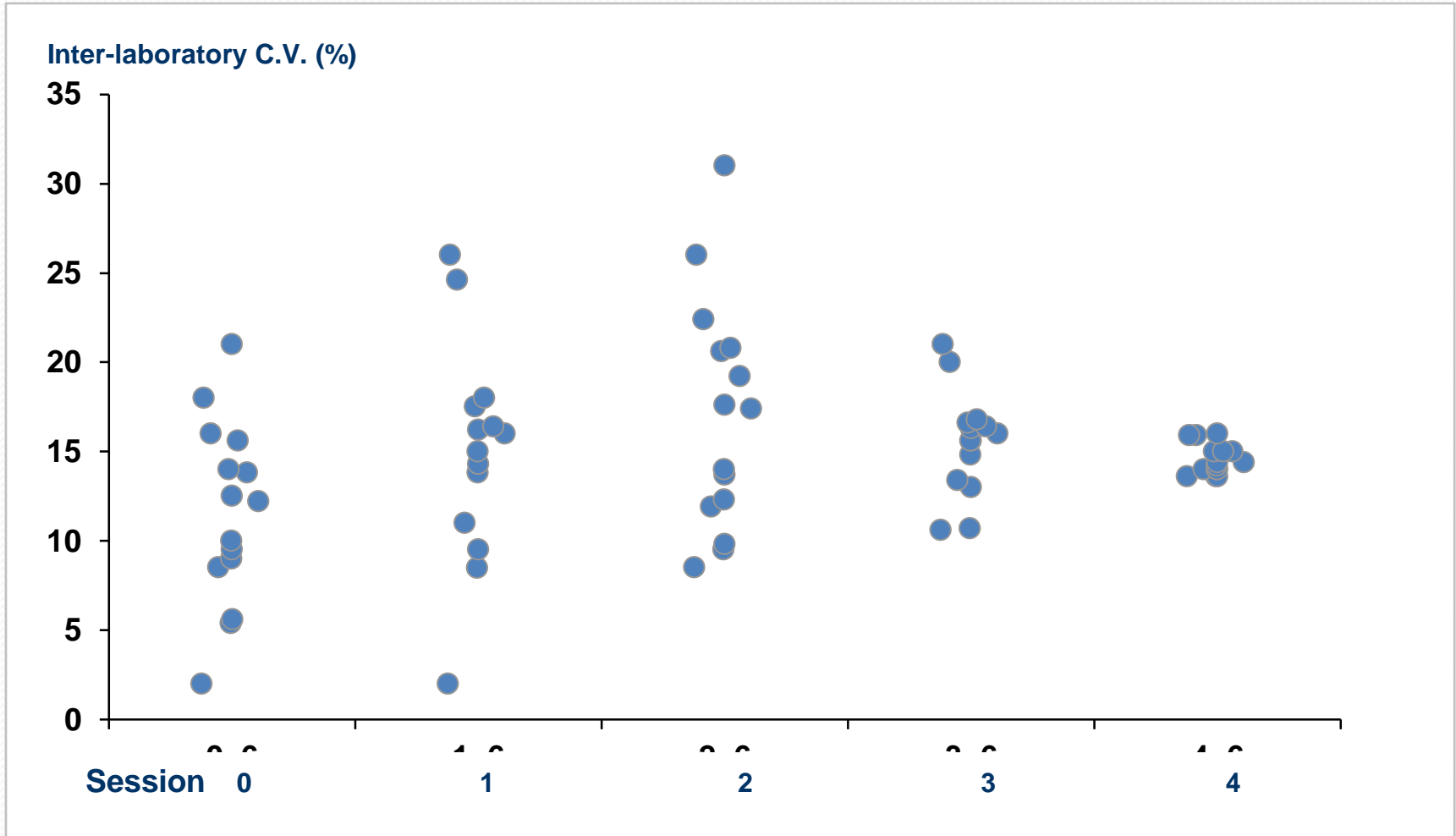
Inter-laboratory C.V. of Sample 6 in Consecutive Sessions



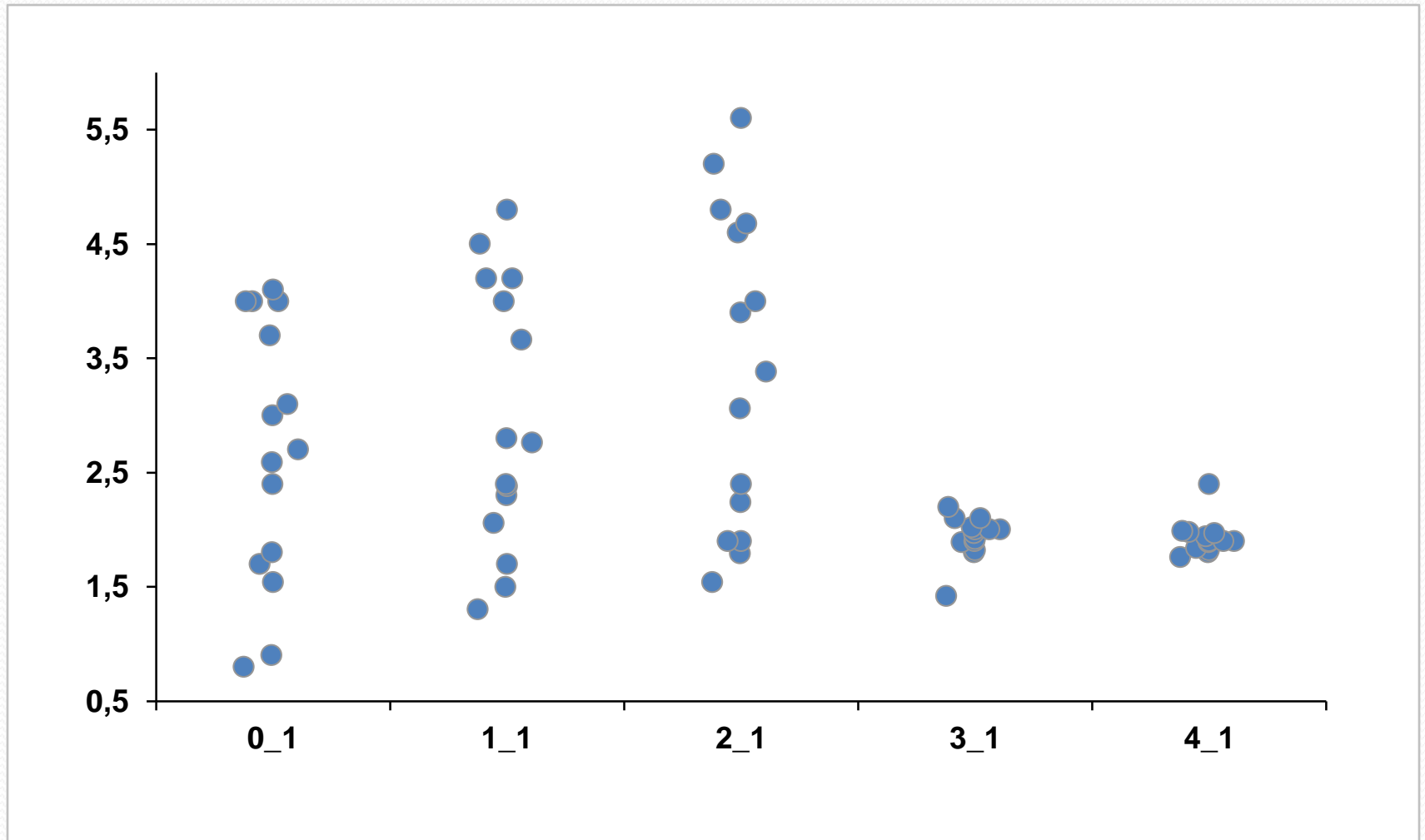
Inter-laboratory C.V. of Sample 6 in Consecutive Sessions



Inter-laboratory C.V. of Sample 6 in Consecutive Sessions



Inter-laboratory C.V. of Sample 1 in Consecutive Sessions



Summary of Workshop Results

Sample no.	U/mL	Session 0	Session 1	Session 2	Session 3	Session 4
1	1.60	35.8%	39.0%	40.7%	9.6%	7.6%
2	0.81	49.0%	69.1%	51.4%	14.1%	5.2%
3	1.40	41.2%	30.2%	34.6%	12.5%	6.4%
4	0.74	69.5%	45.2%	42.8%	14.7%	13.4%
5	1.94	36.0%	41.0%	35.7%	12.8%	12.0%
6	15.4	35.8%	41.3%	38.1%	19.1%	5.8%

Lessons from the Workshop

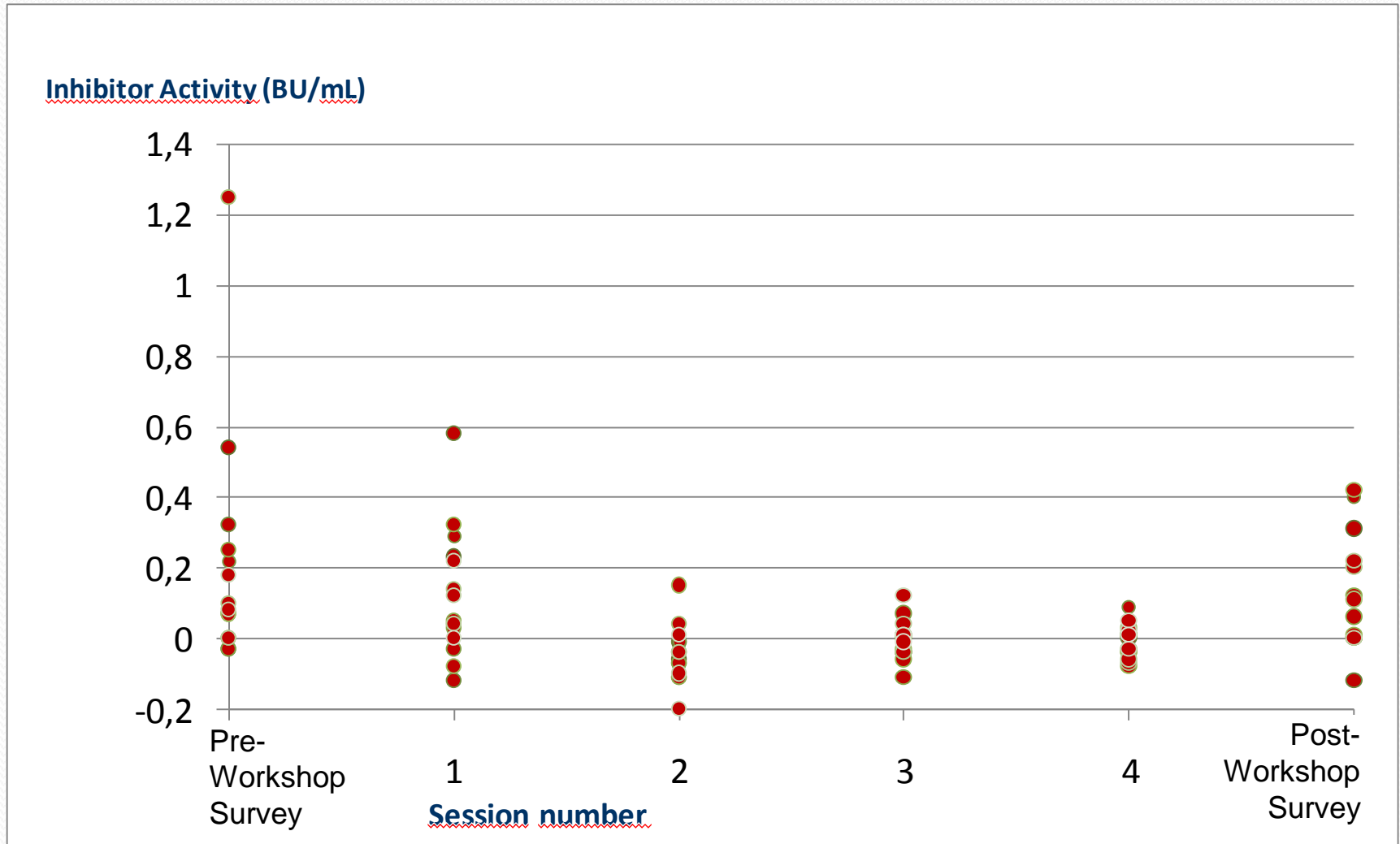
- **Standardization of FVIII inhibitor test, and thus better comparison of test results is possible**
- **(Additional) buffering of normal pool plasma is absolutely necessary to reach a high specificity of the assay**
- **It is absolutely a “must” for individual laboratories to implement all the conditions that are necessary to reach a reliable inhibitor assay, e.g.:**
 - **(Extra-) Buffering of normal pool plasma**
 - **Optimal dilution strategy**
 - **Appropriate control sample**
- **Variations in liquid handling seem to be of minor importance**

Post-workshop survey

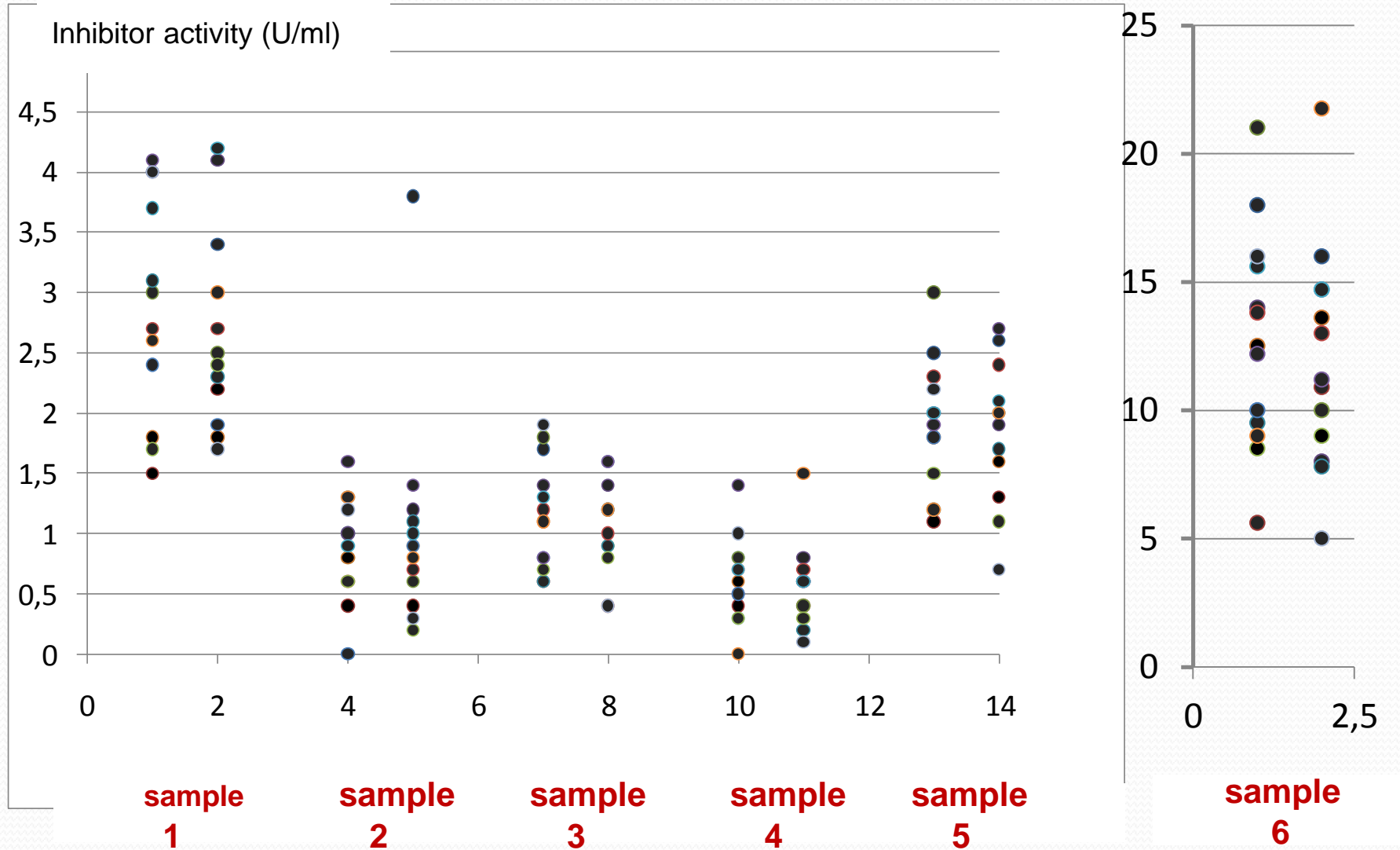
Participants received a report of the workshop and some suggestions how to improve the results of the inhibitor assay.

Thereafter the samples were distributed once again among the 15 laboratories (labels were changed) and results were reported back to ECAT.

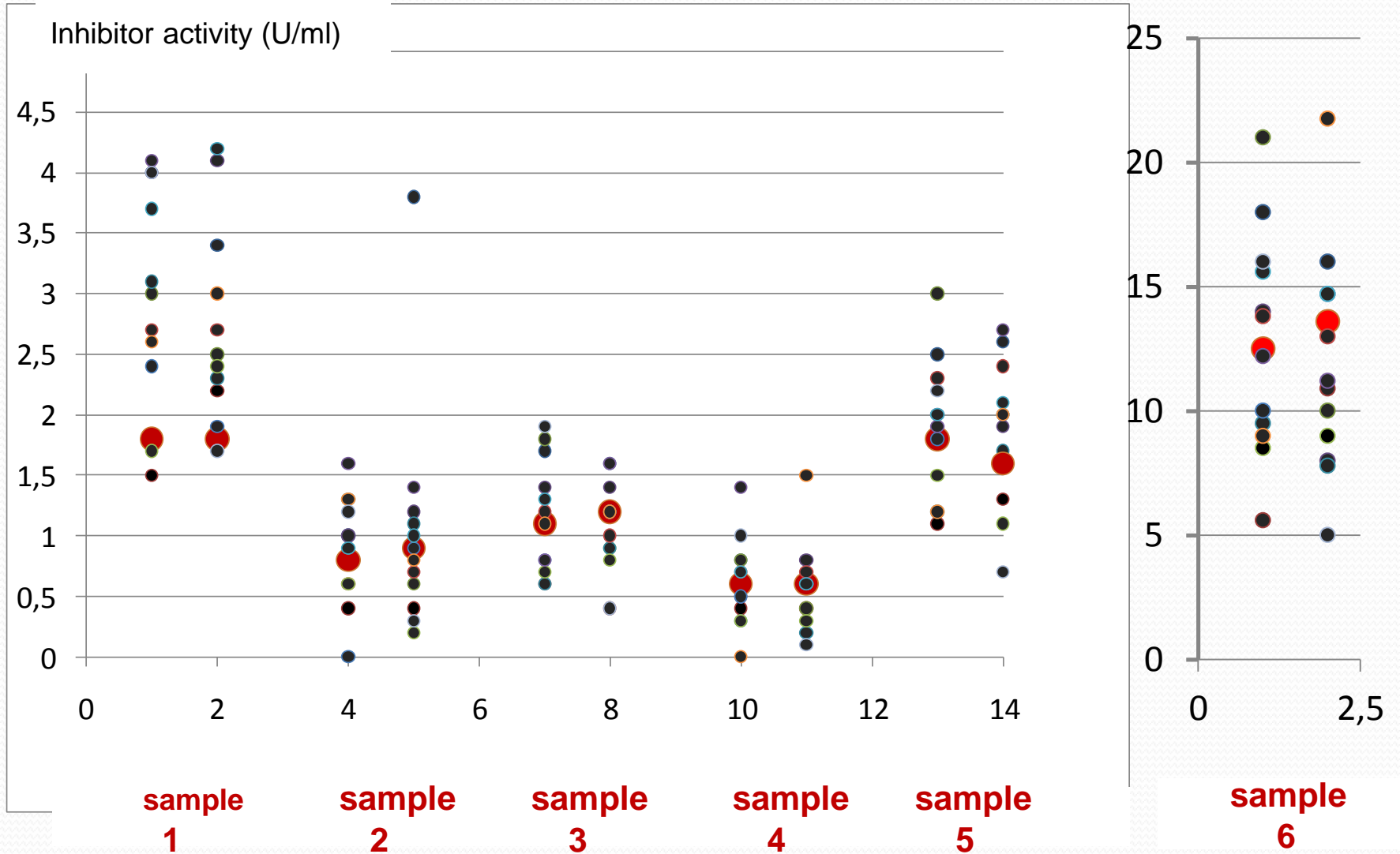
FVIII Inhibitor Activity of Inhibitor Negative Sample in Different Sessions and Post-Workshop



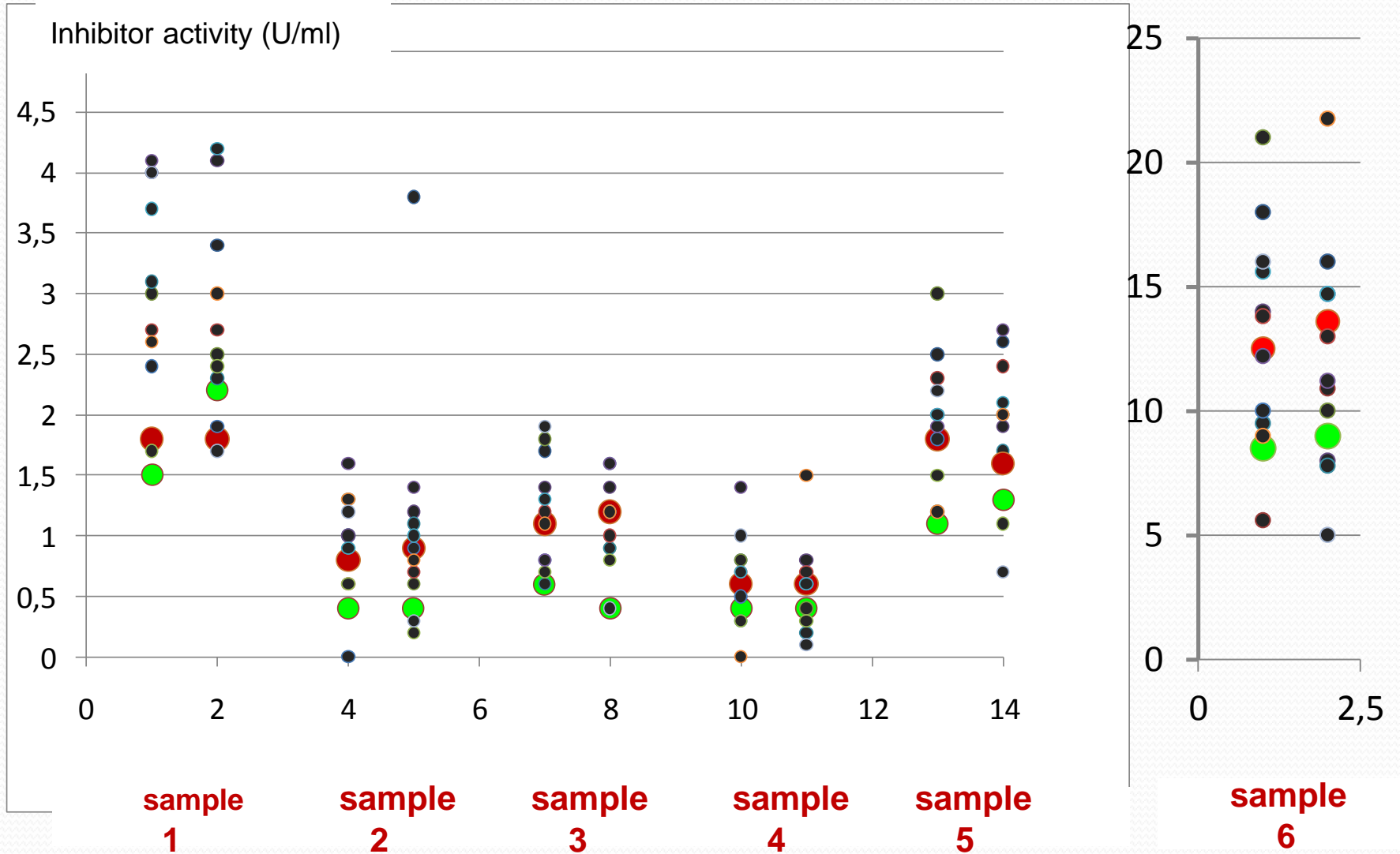
Pre- and post-workshop surveys



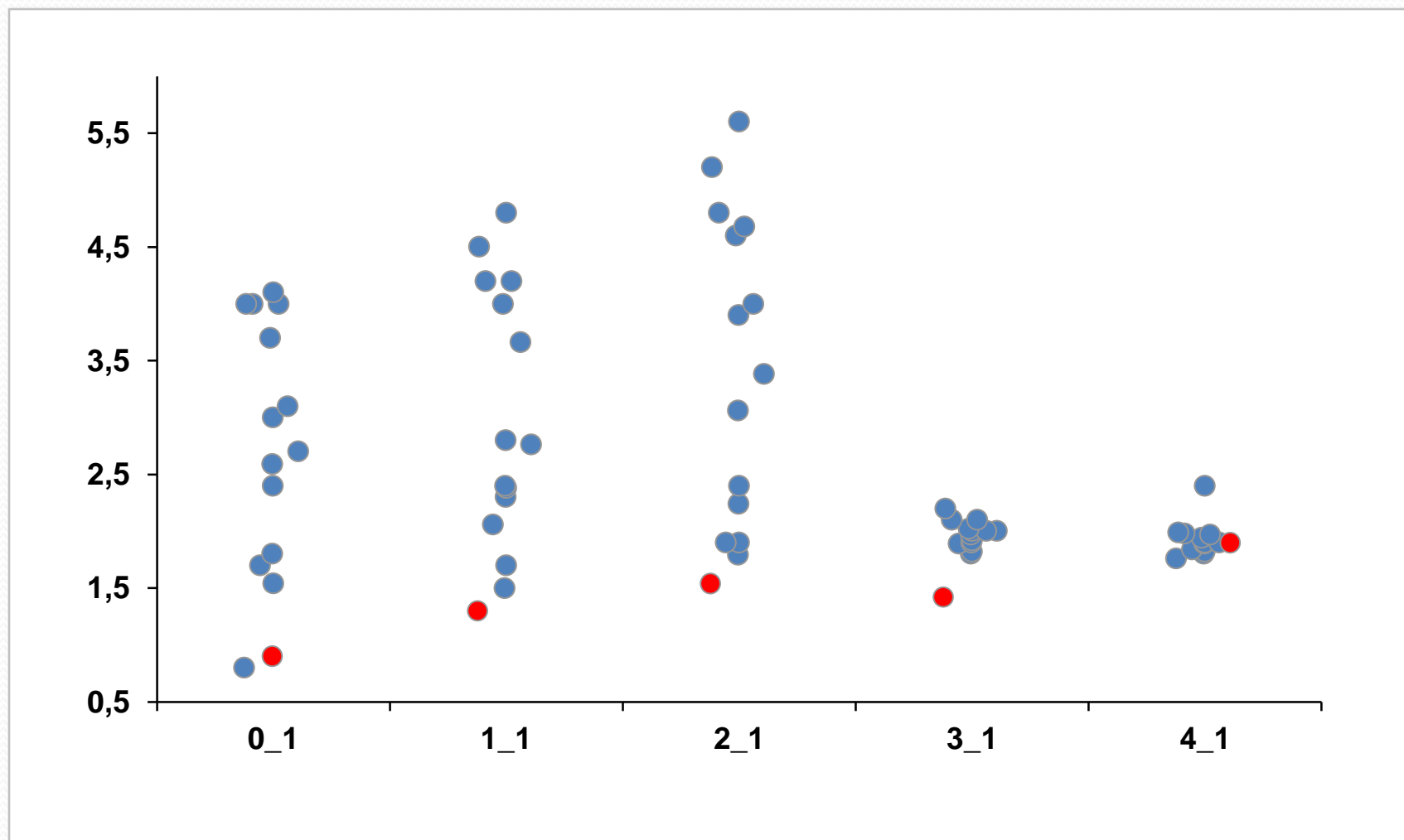
Pre- and post-workshop surveys



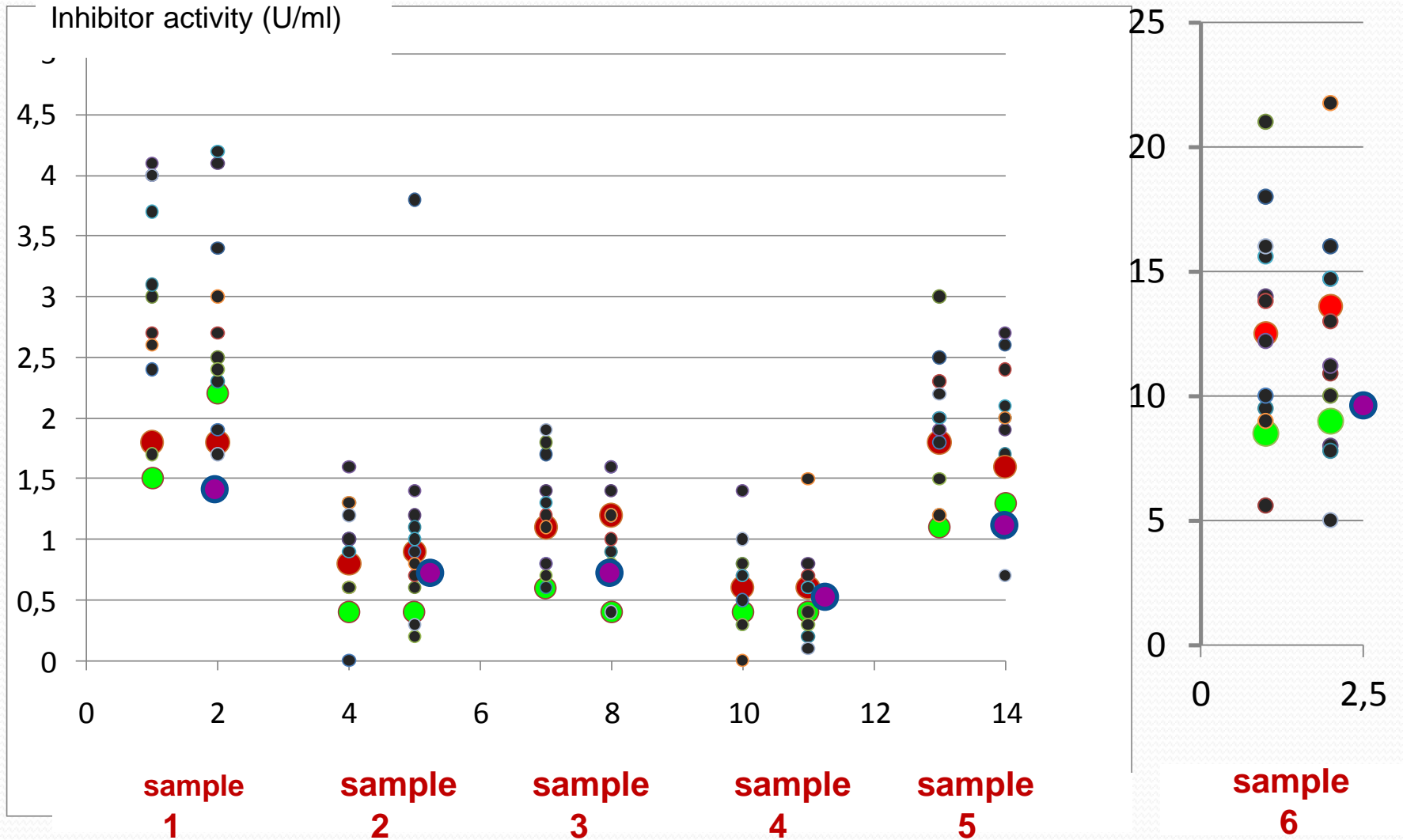
Pre- and post-workshop surveys



Inter-laboratory C.V. of Sample 1 in Consecutive Sessions



Pre- and post-workshop surveys



(Preliminary) Lessons from the Post-Workshop Survey

- The problem of the high inter-laboratory c.v. is rather resistant.
- The causes are multifactorial
- The different types of reagents for FVIII assay may account for at least part of the high c.v.
- Everyone has to realize that **RECOMMENDATIONS** may also be applicable for **YOUR OWN** laboratory.....



Thanks to.....

ECAT foundation for sponsoring the Workshop

Piet Meijer for cooperating in the study

**Petra ter Hark, Clint van Duren, Trix de Boer and
Robert Polenewen for everything**

**All technicians of the Radboud University that participated in the
workshop**

**Roche Diagnostics company for supplying the STA analyzers and
assistance during the workshop**