

# Automated assay-specific statistical evaluation of test run results

Jos Weusten

Bologna, May 9, 2023



# Overview

- Main analyses presented in statistical report
  - What is the performance of the customer over time?
  - How does it relate to results of other customers (peers)?
  - What about different product batches?
  - What about different reagent lots?
  - Provide some overall statistics
- Not an in-depth statistical analysis
  
- Sensitivity analyses for NAT assays: notes on Probit analysis

# Statistical report – page 1

## DataQ Analytics

### Interlaboratory comparison - Statistical Report

**Laboratory:** Sanquin National Screening Service  
**Product:** P0386 SeraQ Alinity V4  
**Assay:** Alinity s HBsAg Reagent

**Manufacturer:** Abbott  
**Marker:** HBsAg  
**Result Unit:** S/CO

Report date: 24 04 2023  
at 14:46

#### Summary statistics with intervals based on LogNormal distribution

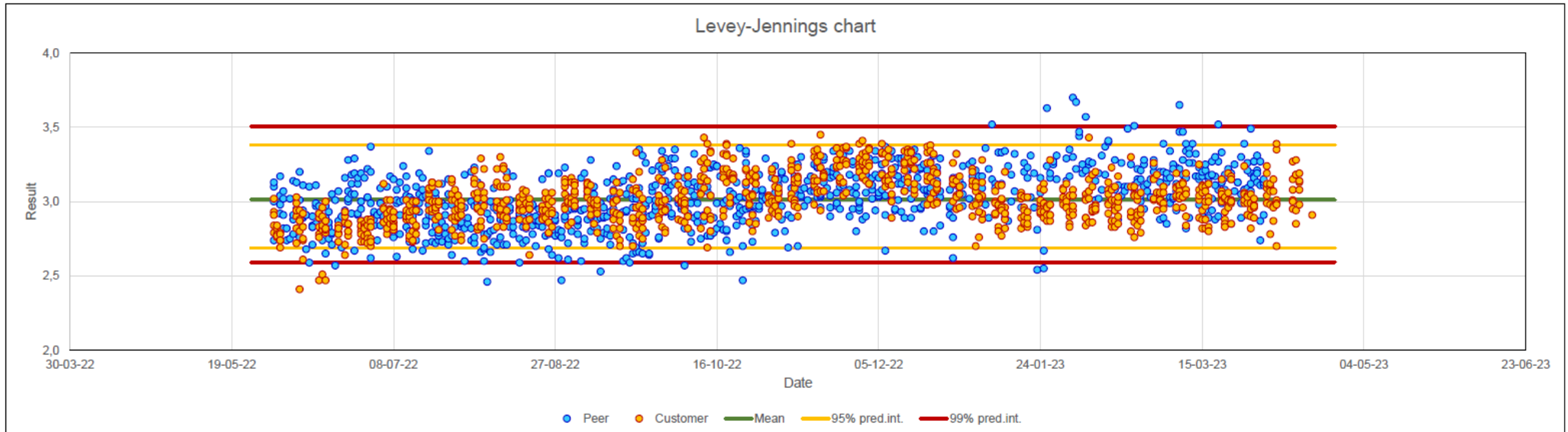
	n	GeoMean	%CV	95% pred.int.		99% pred.int.	
Peer	1014	3,02	6,408	2,66	3,42	2,56	3,56
Customer	974	3,01	5,248	2,72	3,34	2,63	3,45
All	1988	3,01	5,868	2,69	3,38	2,59	3,51

Start date 01-06-22  
End date 18-04-23

#### Violations of Nelson rules by customer

Rule	n	%	Short description
Nr.1	4	0,4	1 value beyond 99% limit
Nr.5	1	0,1	2 consecutive values beyond same 95% limit
Nr.3	2	0,2	6 consecutive values continuously increasing or decreasing

56 observations with value <0,1 removed  
2 observations with value >6 removed



# Statistical report – page 2

## Customer results violating Nelson rules

Rule 1: Individual value beyond 99% prediction limit

Equipment	Date	Unit Ratio
AS1051	09-06-22	2,41
AS1051	15-06-22	2,47
AS1051	16-06-22	2,51
AS1051	17-06-22	2,47

Indication of incidental outliers.

Expected frequency if process is under control: 1%

Rule 5: At least 2 consecutive values beyond the same 95% prediction limit

Equipment	Date	Unit Ratio
AS1051	13-10-22	3,39

Indication of potential shift in the process mean

Rule 3: At least 6 consecutive values are continuously increasing (or decreasing)

Equipment	Date	Unit Ratio
AS1051	02-06-22	2,76
AS1051	03-08-22	2,81

Indication of potential drift in the process.

Expected frequency if process is under control: about 0.1%

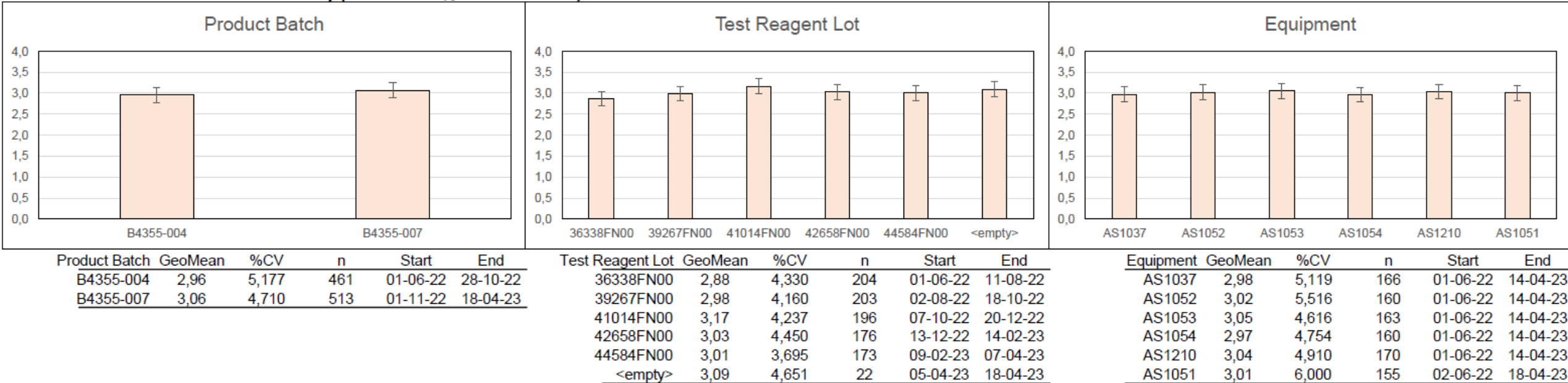
## Summary reported on page 1

### Violations of Nelson rules by customer

Rule	n	%	Short description
Nr.1	4	0,4	1 value beyond 99% limit
Nr.5	1	0,1	2 consecutive values beyond same 95% limit
Nr.3	2	0,2	6 consecutive values continuously increasing or decreasing

# Statistical report – page 3

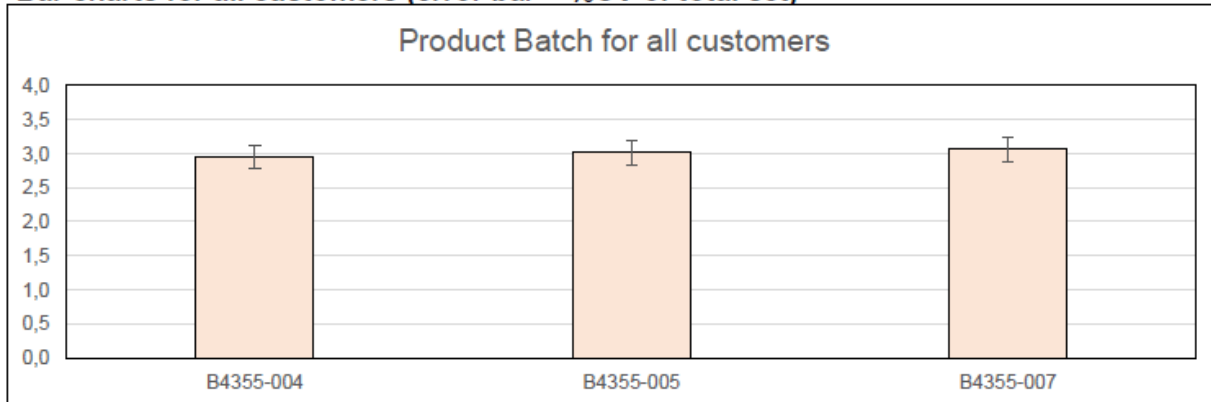
Bar charts for results of customer only (error bar = %CV of total set)



Sequence in bar charts given by first date the product batch / test reagent lot / equipment appears in the data set

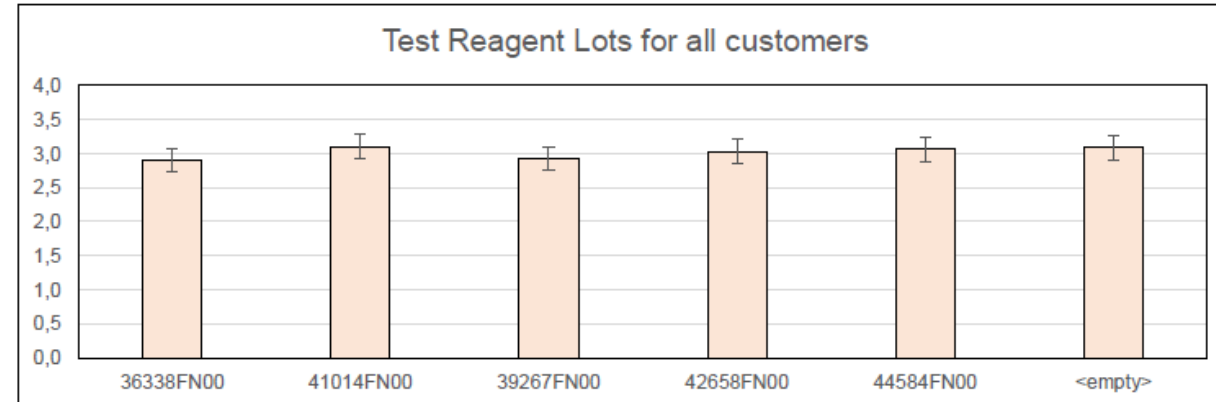
# Statistical report – page 4

Bar charts for all customers (error bar = %CV of total set)



Product Batch	GeoMean	%CV	n	Start	End
B4355-004	2,96	5,177	461	01-06-22	28-10-22
B4355-005	3,02	6,408	1014	01-06-22	03-04-23
B4355-007	3,06	4,710	513	01-11-22	18-04-23

Apparently lot 005 not used by customer



Test Reagent Lot	GeoMean	%CV	n	Start	End
36338FN00	2,90	4,809	330	01-06-22	11-08-22
41014FN00	3,11	5,606	674	13-06-22	28-02-23
39267FN00	2,93	5,137	487	09-07-22	18-10-22
42658FN00	3,03	4,450	176	13-12-22	14-02-23
44584FN00	3,06	4,811	299	09-02-23	07-04-23
<empty>	3,09	4,651	22	05-04-23	18-04-23

Sequence in bar charts given by first date the product batch / test reagent lot appears in the data set

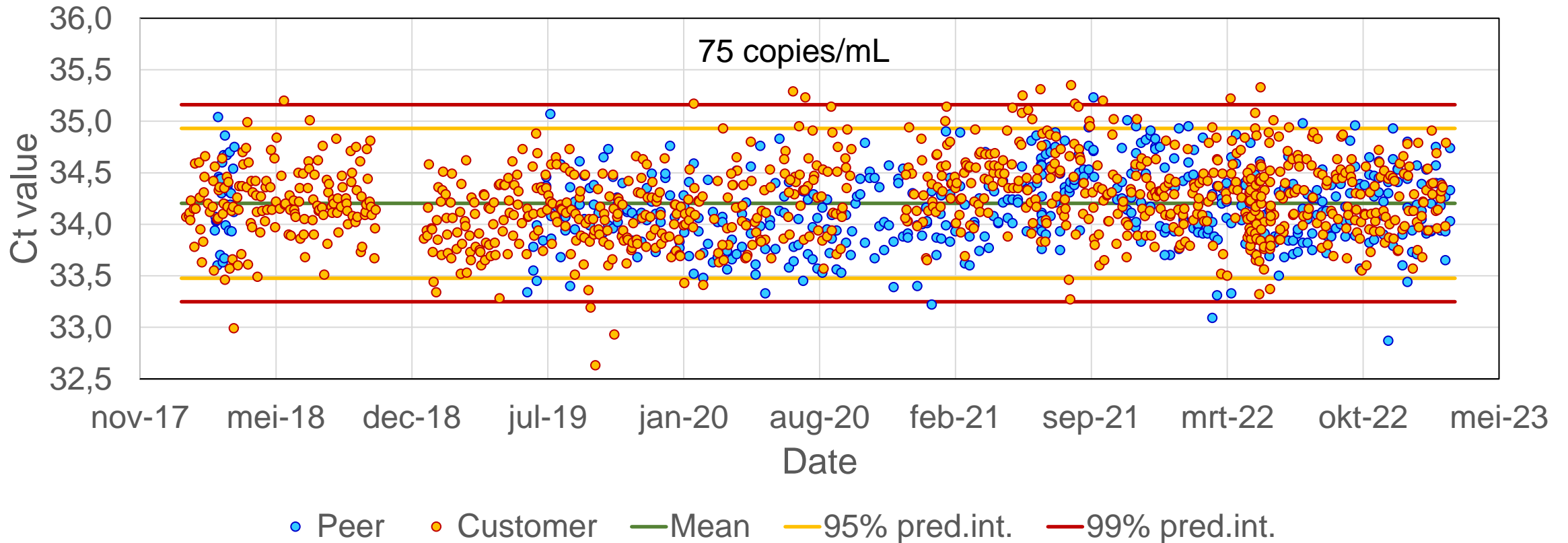
Comparison equipment over multiple customers is not relevant

# Different distributions

- Depending on the assay, the data follow different distributions
- PCR tests, with Ct value as response
  - Typically a Normal distribution
  - Summary statistics: mean, SD, limits of prediction intervals
- Immunoassays (CLIA, ELISA)
  - Typically a log-normal distribution
  - Summary statistics calculated using log-transformed values
  - Summary statistics reported as geometric mean, %CV, limits of prediction intervals
- Other (TMA)
  - Undefined distribution
  - Summary statistics: mean, median and SD and ranges covered by 95% and 99% of the data (based on 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles and 0.5<sup>th</sup> and 99.5<sup>th</sup> percentiles)

# HIV-1 RNA Ct values in cobas MPX assay on P0273 ViraQ Control (Normal)

(data Jan 2018 – Feb 2023 - Sanquin National Screening Service ● and Flemish Red Cross ●)

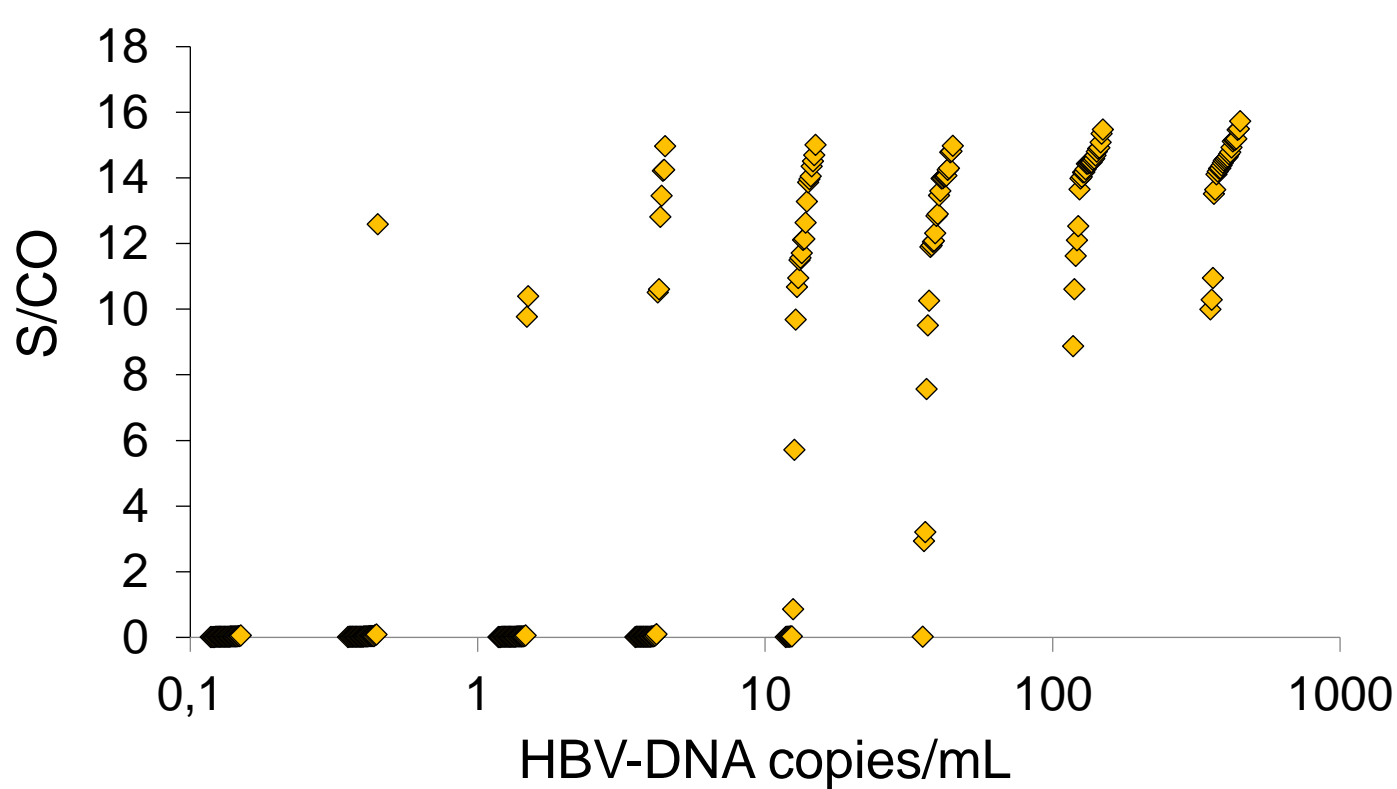


## Summary statistics with intervals based on Normal distribution

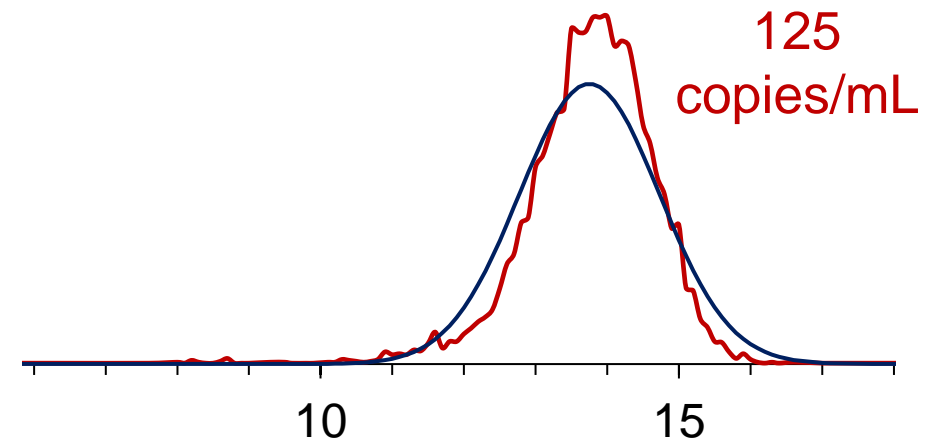
	n	Mean	StDev	95% pred.int.		99% pred.int.	
Peer	514	34,17	0,369	33,44	34,89	33,21	35,12
Customer	946	34,22	0,370	33,50	34,95	33,27	35,18
All	1460	34,20	0,371	33,48	34,93	33,25	35,16



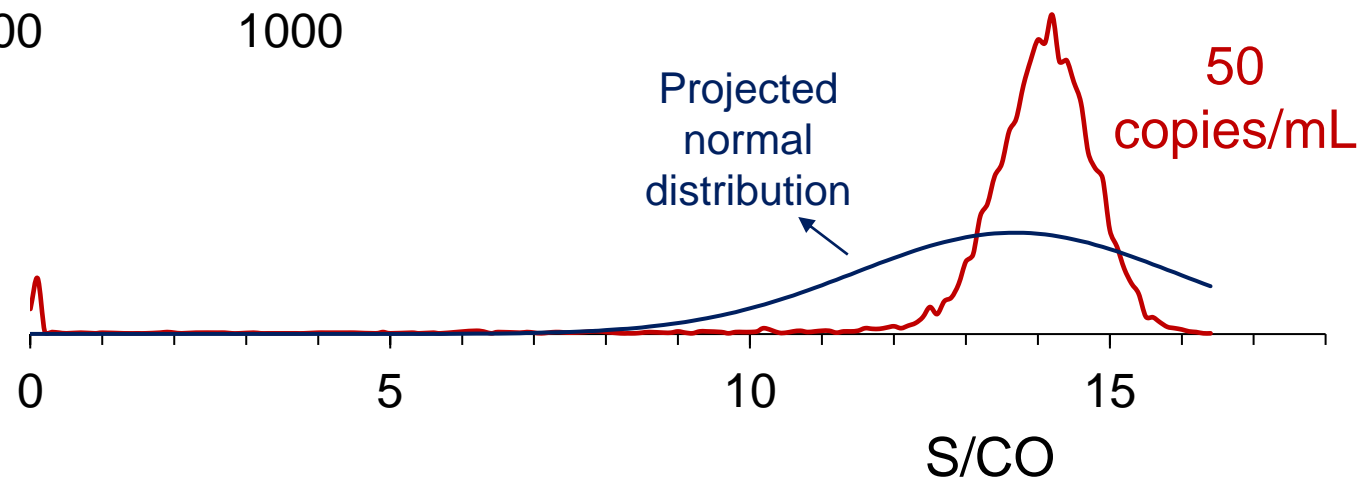
# Nonparametric distribution of S/CO values in Ultrio Elite assay on HBV-DNA standard dilution in ViraQ Check 125 and Trend 50 Control



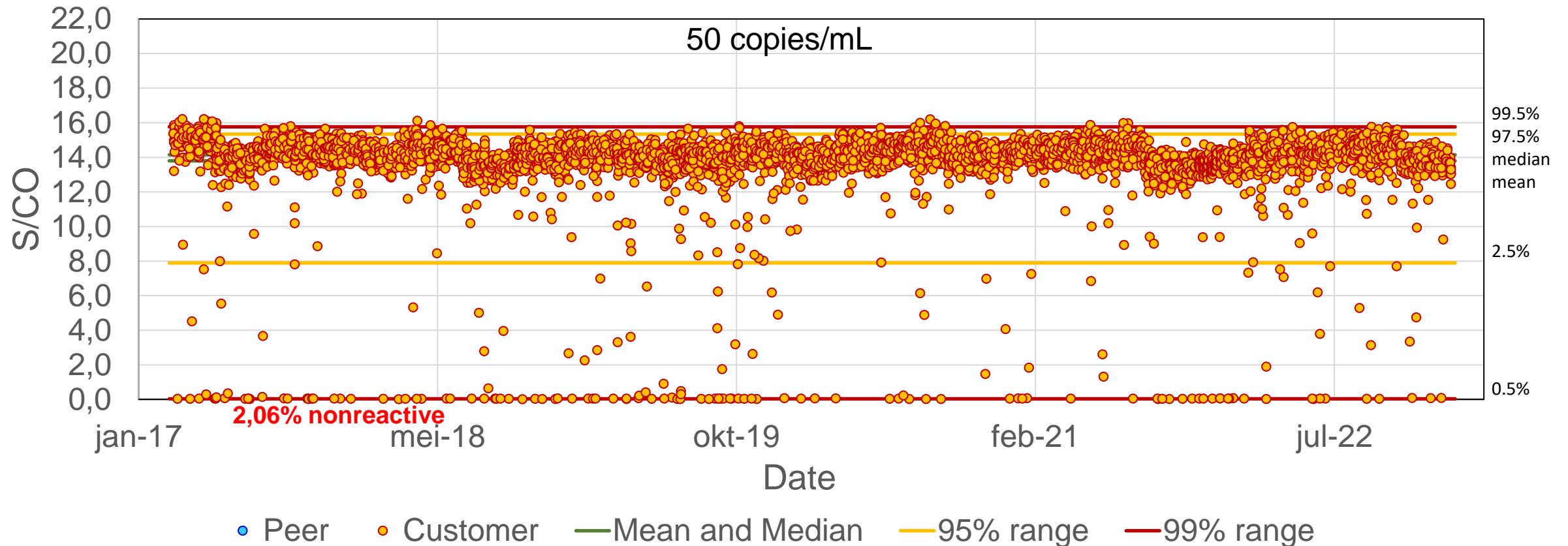
P0065 ViraQ HBV Check 125 (n=7291)



P0154 ViraQ HBV Trend 50 (n=7743)



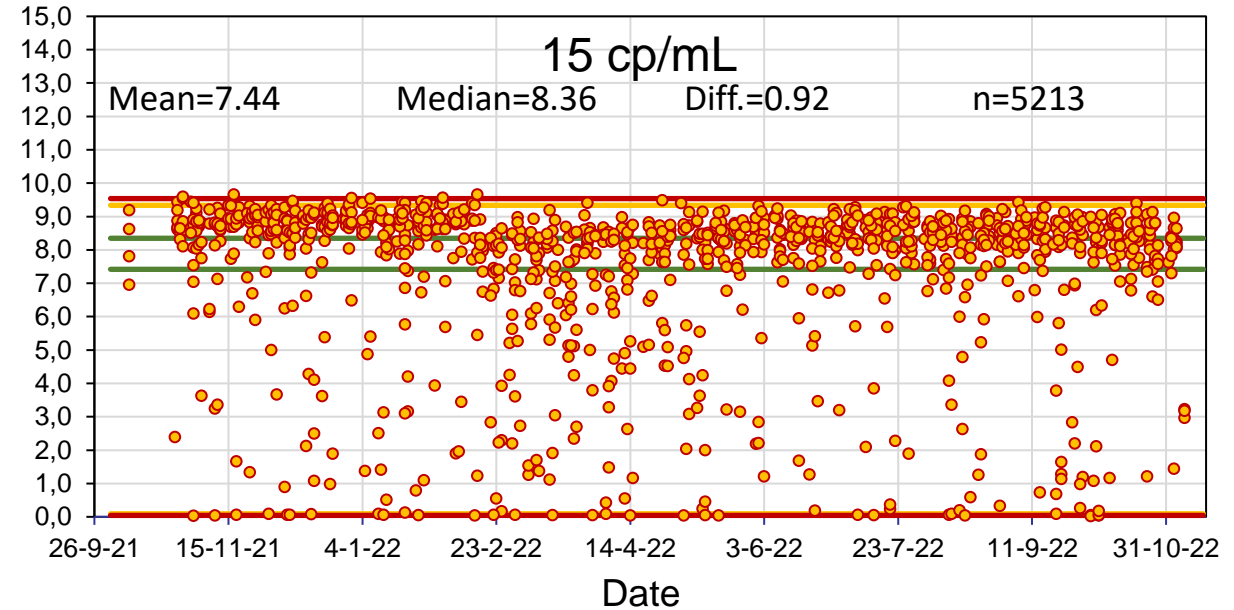
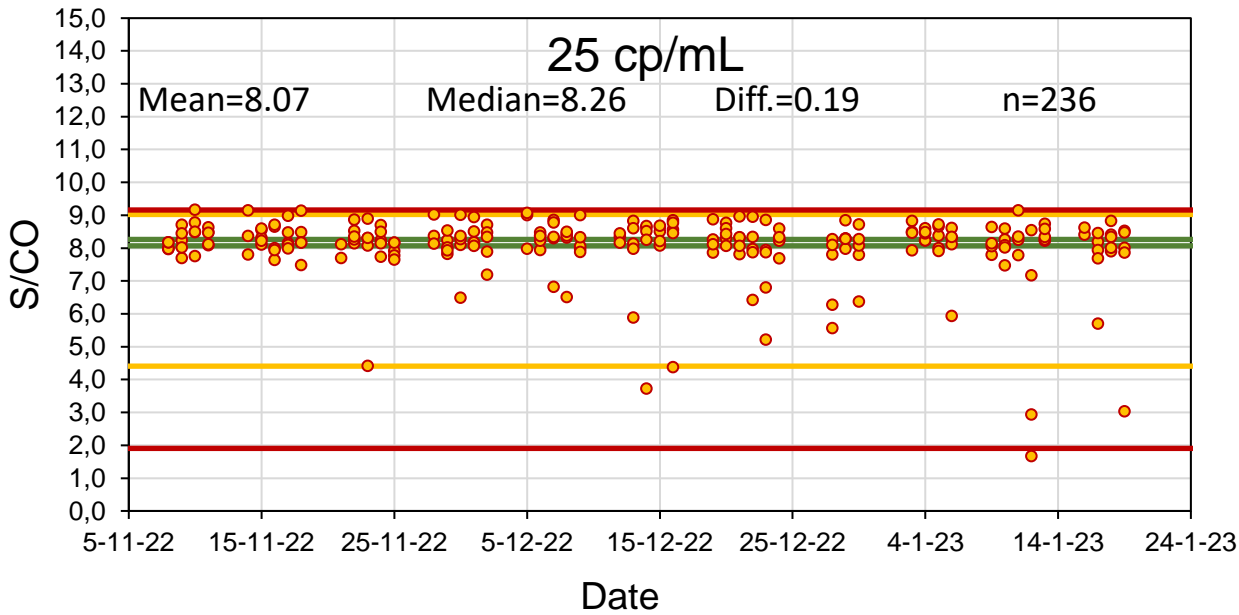
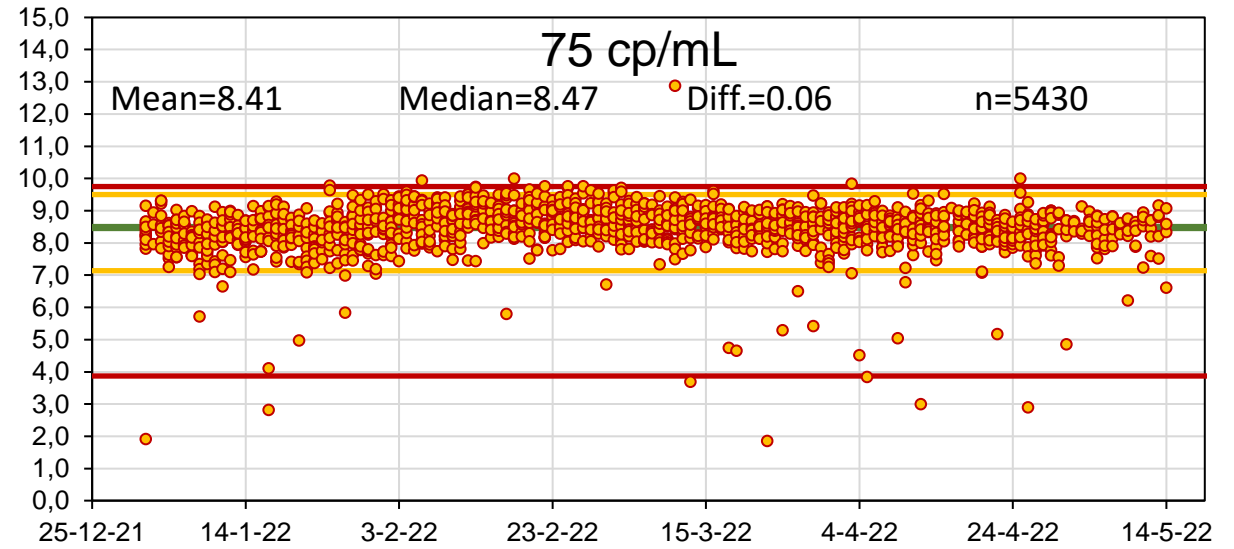
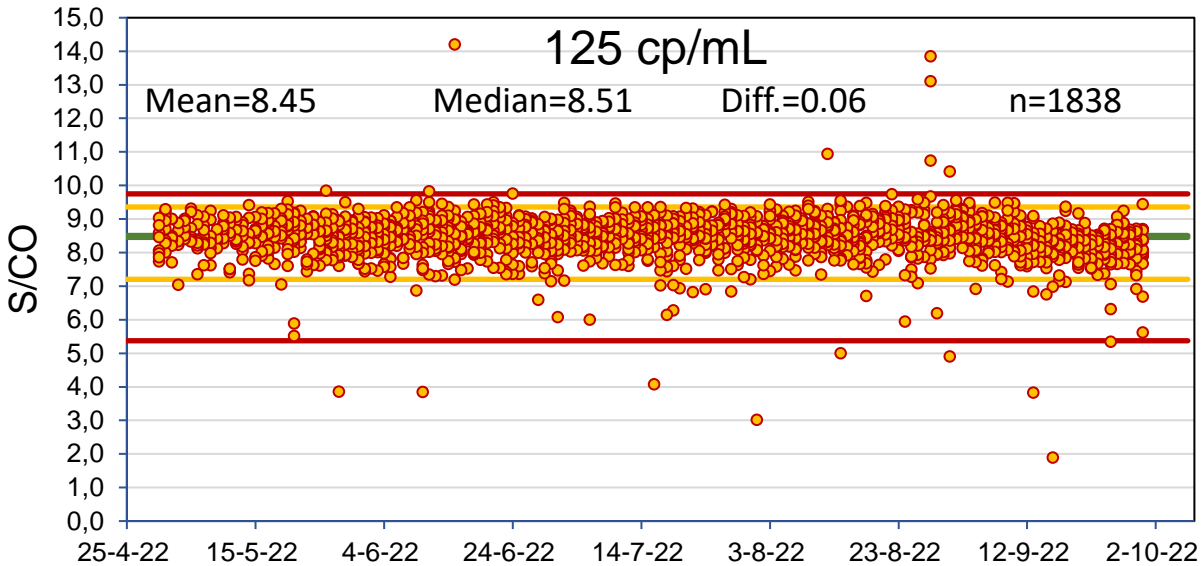
# HBV-DNA S/CO values in Ultrio Elite Assay on P0154 ViraQ Trend 50 Control (Data 2017 - 2023 of Irish Blood Transfusion Service (IBTS)) – distribution-free analysis



## Summary statistics with non-parametric intervals

	n	Mean	StDev	Median	95% range	99% range
All	6466	13,79	2,155	14,15	7,89 15,34	0,04 15,76

# Impact of HCV-RNA concentration in ViraQ Controls on Ultrio Elite S/CO value distribution and Levey-Jennings Chart parameters

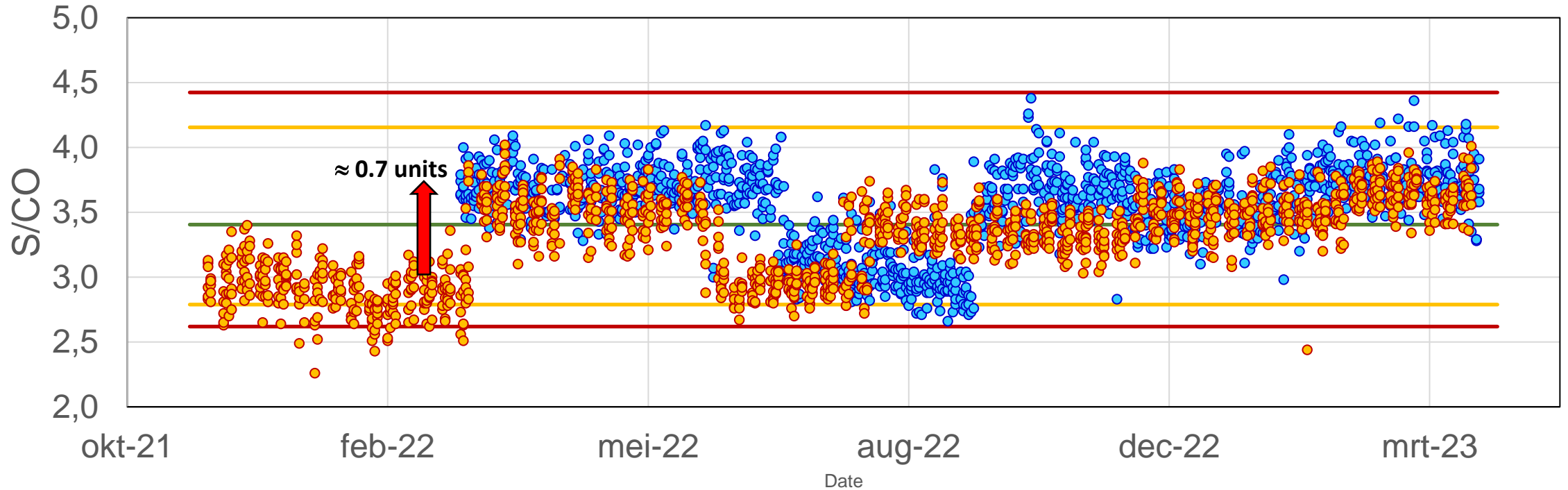


● Peer   ● Customer   — Mean and Median   — 95% range   — 99% range

● Peer   ● Customer   — Mean and Median   — 95% range   — 99% range

# Performance of Alinity HIV combo assay on P0386 SeraQ Control

(data 2022 - 2023 - Sanquin National Screening Service ● and Citta della salute e della Scienze di Torino ●)



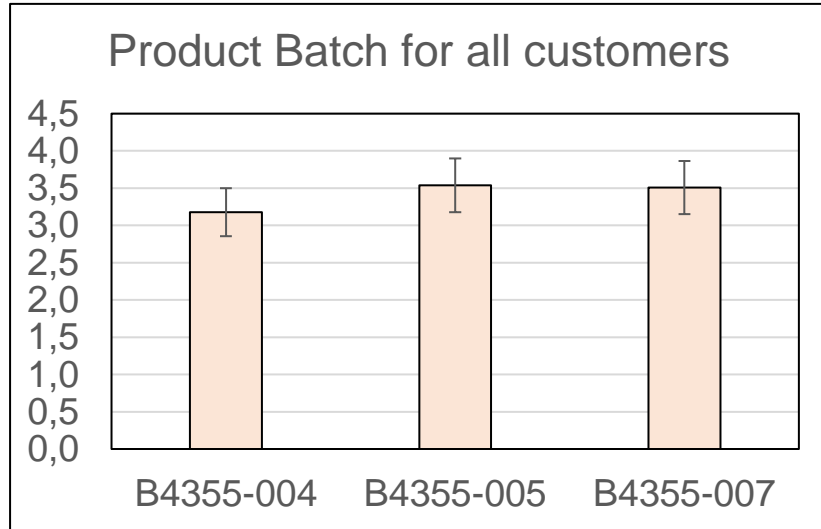
● Peer ● Customer — Mean — 95% pred.int. — 99% pred.int.

## Summary statistics with intervals based on LogNormal distribution

	n	GeoMean	%CV	95% pred.int.		99% pred.int.	
Peer	1407	3,54	9,148	2,96	4,23	2,79	4,48
Customer	1454	3,28	9,755	2,71	3,97	2,55	4,22
All	2861	3,40	10,193	2,79	4,15	2,62	4,42

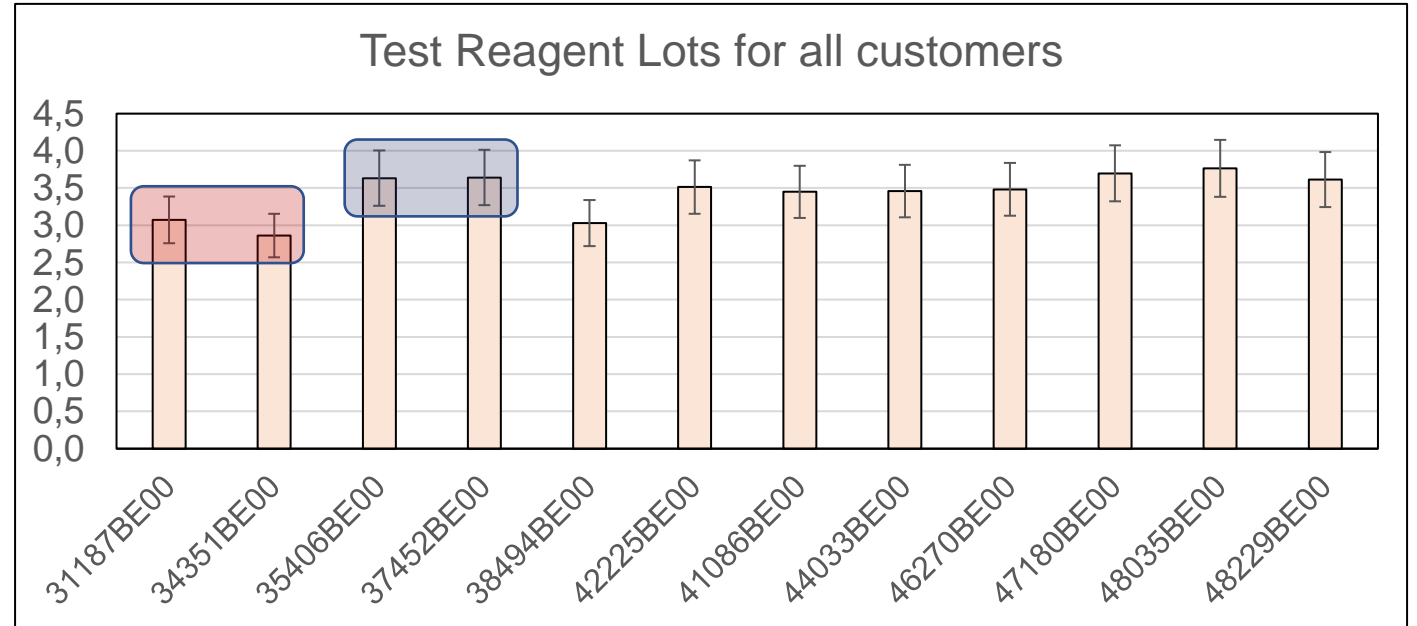
# Geomean S/CO values of Alinity HIV Combo assay reagent lots on P0386 SeraQ Control batches

S/CO



Product Batch	Geo Mean	%CV	n	Start	End
B4355-004	3,18	9,801	984	01-12-21	28-10-22
B4355-005	3,54	9,148	1407	08-03-22	03-04-23
B4355-007	3,51	5,187	470	01-11-22	31-03-23

$3,54 - 3,18 = 0,36$

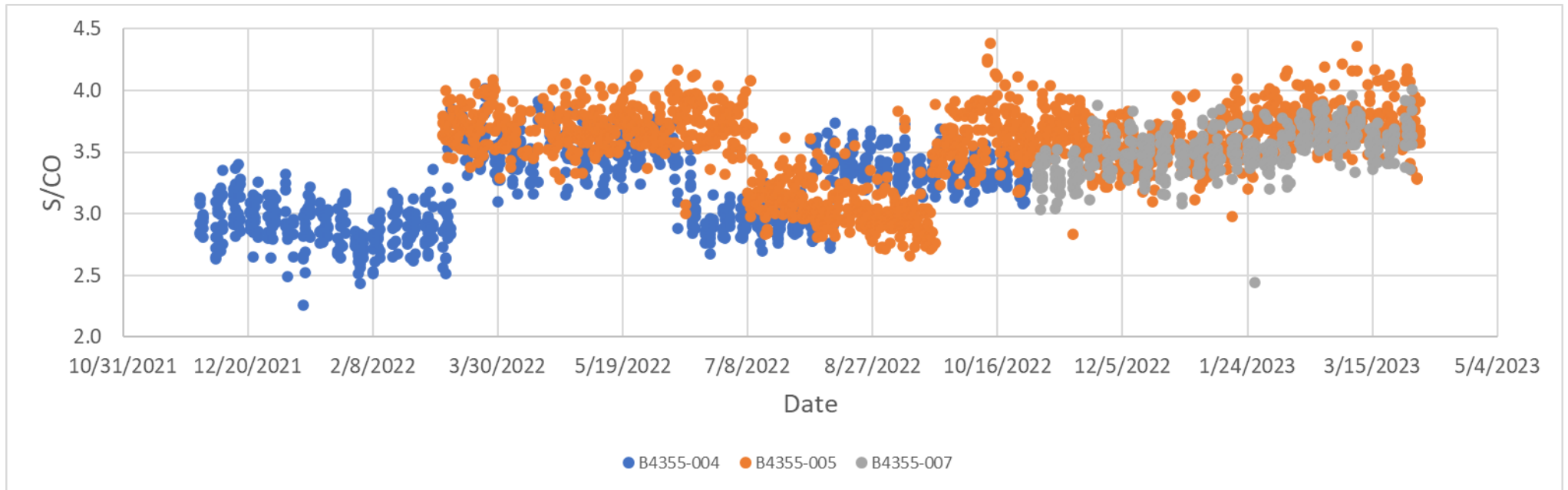


Test Reagent Lot	GeoMean	%CV	n	Start	End
31187BE00	3,07	9,810	164	01-12-21	17-03-23
34351BE00	2,86	5,730	172	13-01-22	11-03-22
35406BE00	3,63	5,025	328	08-03-22	10-05-22
37452BE00	3,64	5,323	345	15-04-22	06-07-22
38494BE00	3,03	6,452	439	10-06-22	16-09-22
42225BE00	3,51	6,802	895	13-06-22	28-02-23
41086BE00	3,45	5,451	91	17-09-22	20-12-22
44033BE00	3,46	4,316	84	13-12-22	11-01-23
46270BE00	3,48	5,228	96	10-01-23	14-02-23
47180BE00	3,70	3,902	125	09-02-23	12-03-23
48035BE00	3,76	4,980	67	13-03-23	31-03-23
48229BE00	3,61	4,681	55	21-03-23	03-04-23

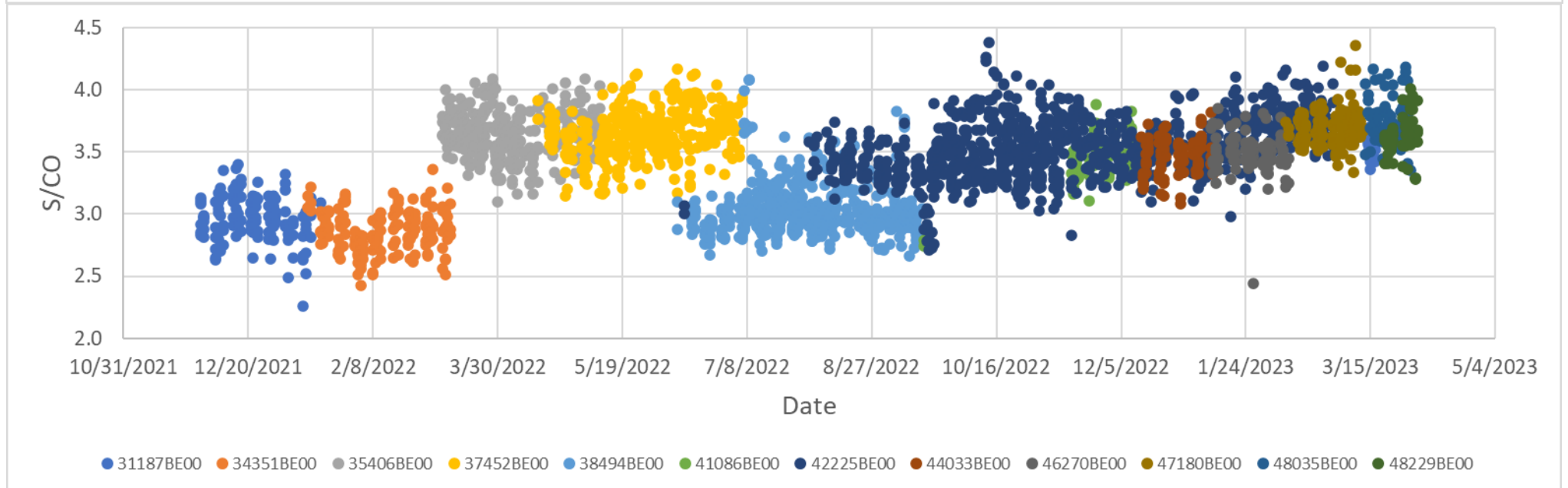
Error bars represent the %CV as derived from the total data set

# Geomean S/CO values of Alinity HIV Combo assay reagent lots on P0386 SeraQ Control batches

Results versus date identified by Product Batch



Results versus date identified by Test Reagent Lot



# Conclusion

- We generate simple graphical overviews over the results over time
- Qualitatively it is easy to detect
  - The overall performance of the customer in terms of consistency
  - Differences between customer and peers
  - Potential differences between product batches, test reagent lots and instruments
- The differences in statistical properties are taken into account
  - Normal / lognormal / distribution-free
- We provide relevant summary statistics
  - Overall level, overall variability, 95% and 99% ranges; Nelson rules
- It is a valuable addition for both customers and





# Notes on Probit analyses

- To assess the 50% and 95% hit rate concentrations of a NAT assay, probit analysis is the standard technique
- The probit model is an empirical model
- The probit model implies symmetry of the curve → is this real?  
(the logistic curve has the same property)
- Reponse is number of positives in a number of samples  
→ just 'pos' and 'neg', so little information in a single result
- Deviation from the model is difficult to assess in case the number of observations is relatively small
- Now we have many data (often >100 replicates per concentration), allowing more detailed analyses



# Probit analyses

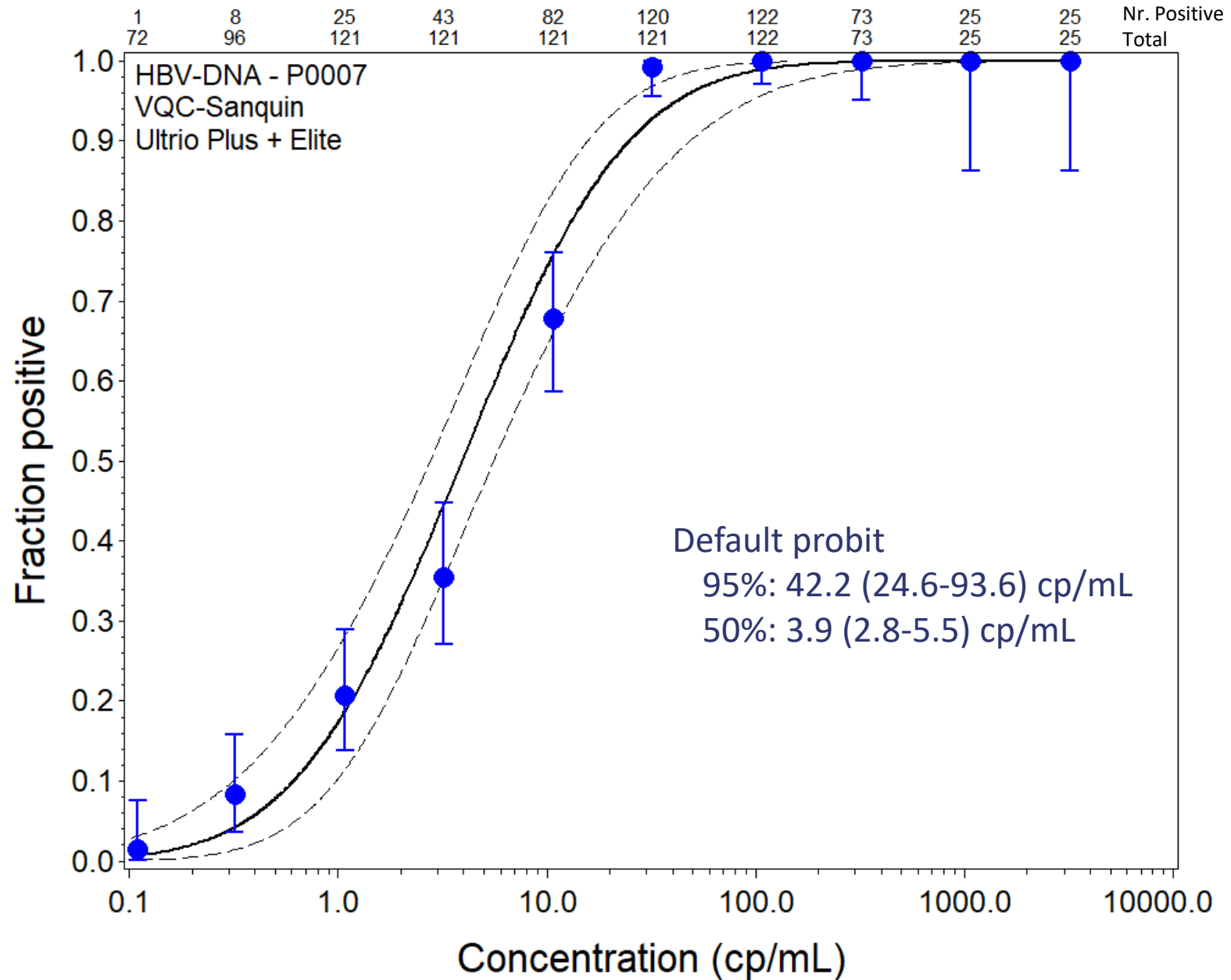
## Default model

The probit curve is perfectly symmetric around the point corresponding to 50% positivity rate

Overall fit is not satisfactory

Relatively high positivity rates at low concentrations affect the estimated 95% hit rate concentration

The estimated 95% hit rate concentration is biased



# Probit analyses

## Default model

## data selection

### Considerations

- Interest focuses on the upper range
- The model is purely empirical

### Therefore:

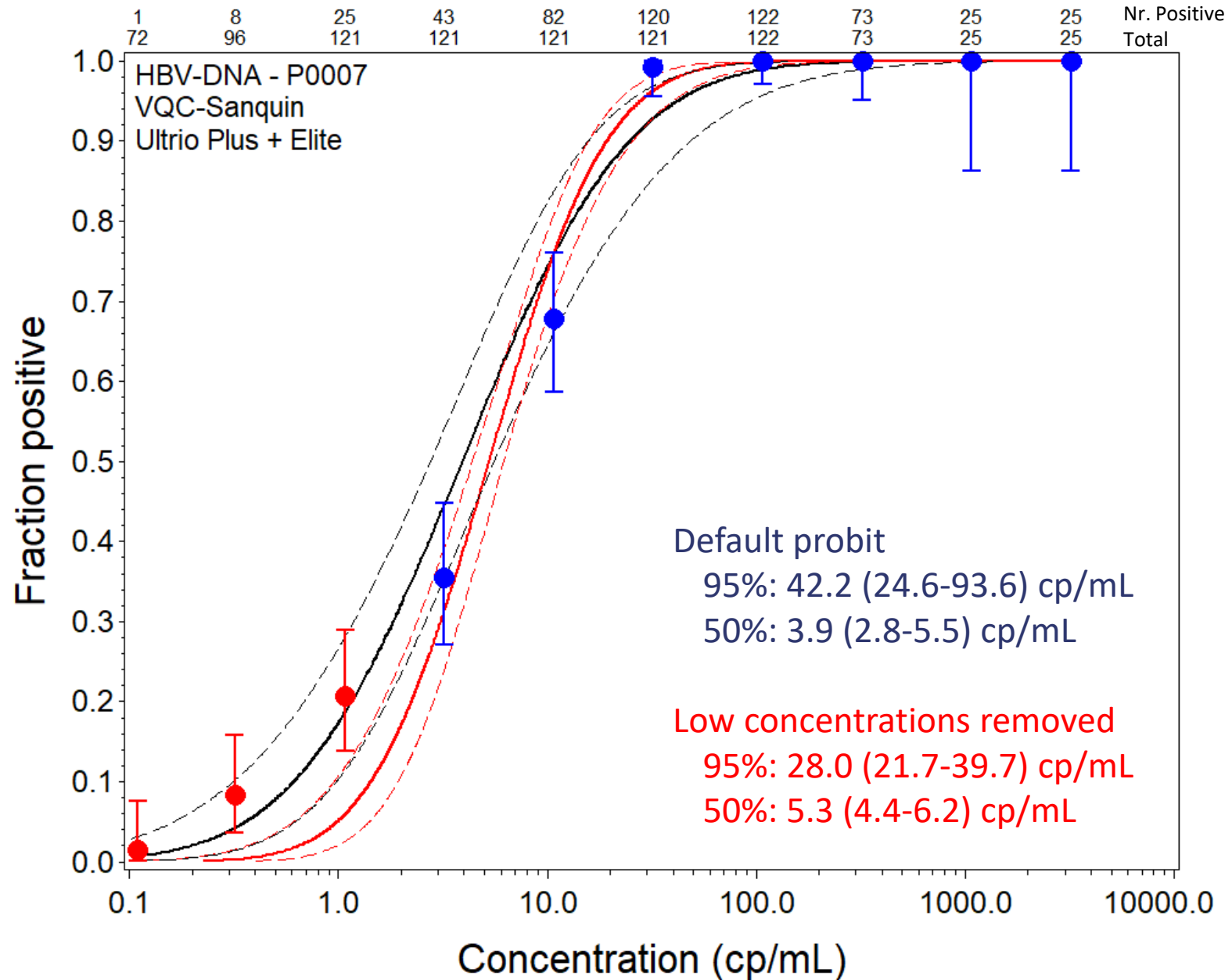
- Remove the lowest concentrations but make sure the observed hit rates cover the 50%-95% range

### Pros:

- Better fit in the high range
- Less biased estimate for 95% hit rate concentration

### Cons:

- Data selection



# Probit analyses

## New link

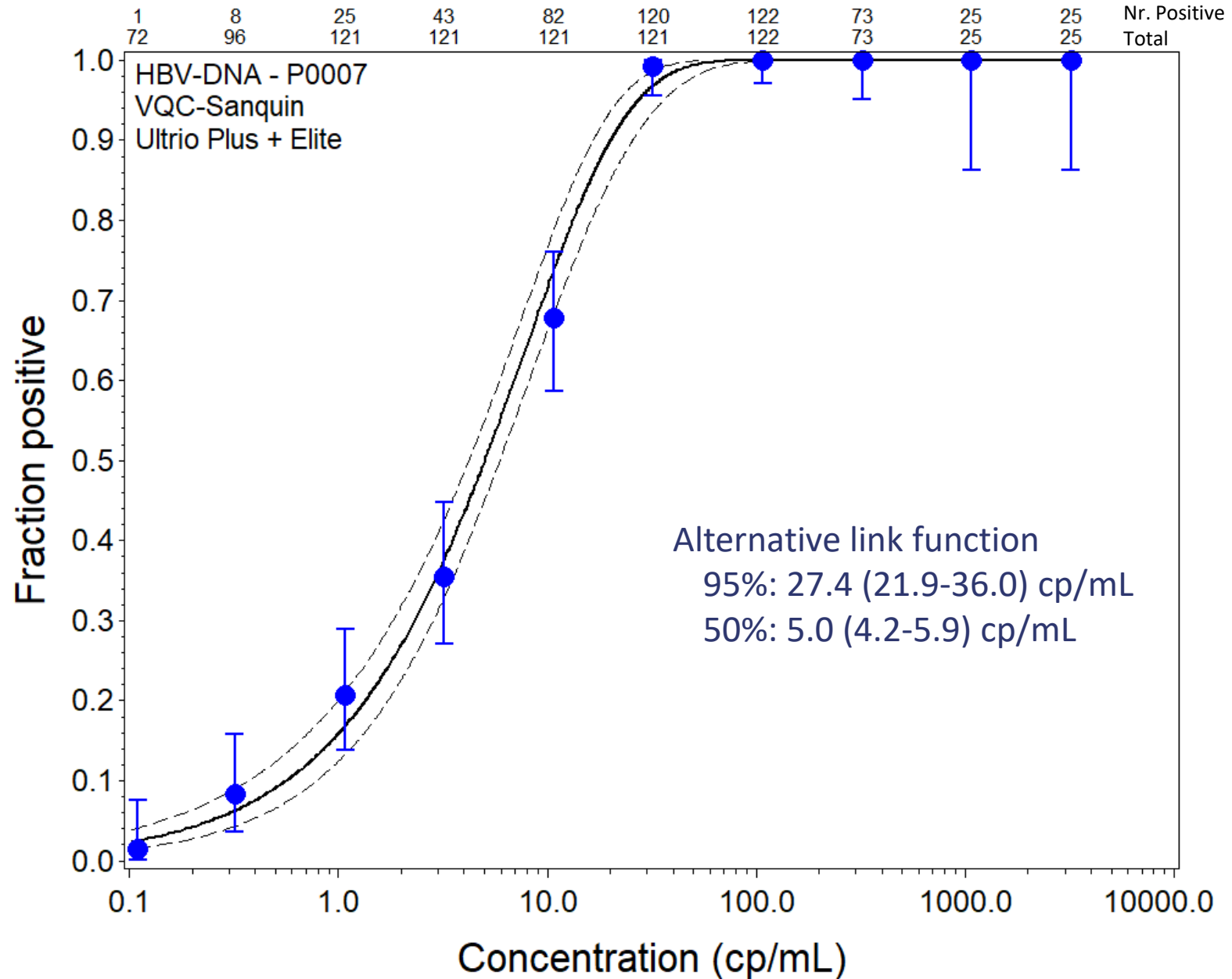
We propose an alternative link function, also empirical

The cumulative distribution function of an extreme value distribution is used rather than of the normal distribution

The model is clearly more convincing over the whole range

Therefore:

- less biased estimates for 50% and 95% hit rate concentrations
- No (arbitrary) data selection needed



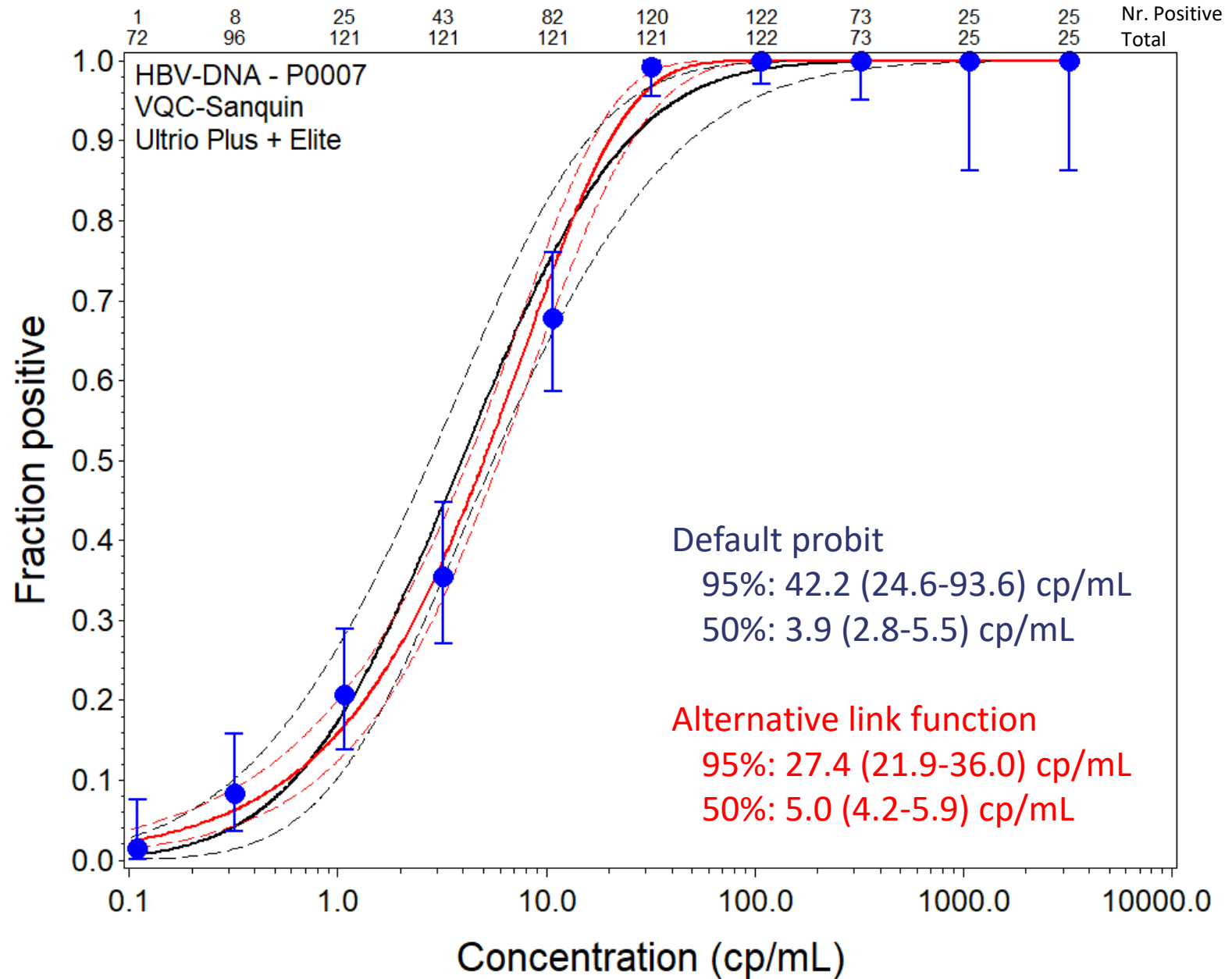
# Probit analyses default model vs alternative link

Overall the quality of the fit with the alternative link function is much better than the default probit

Fit quality statistic (the log likelihood) is considerably better (difference >9)

Confidence intervals much narrower

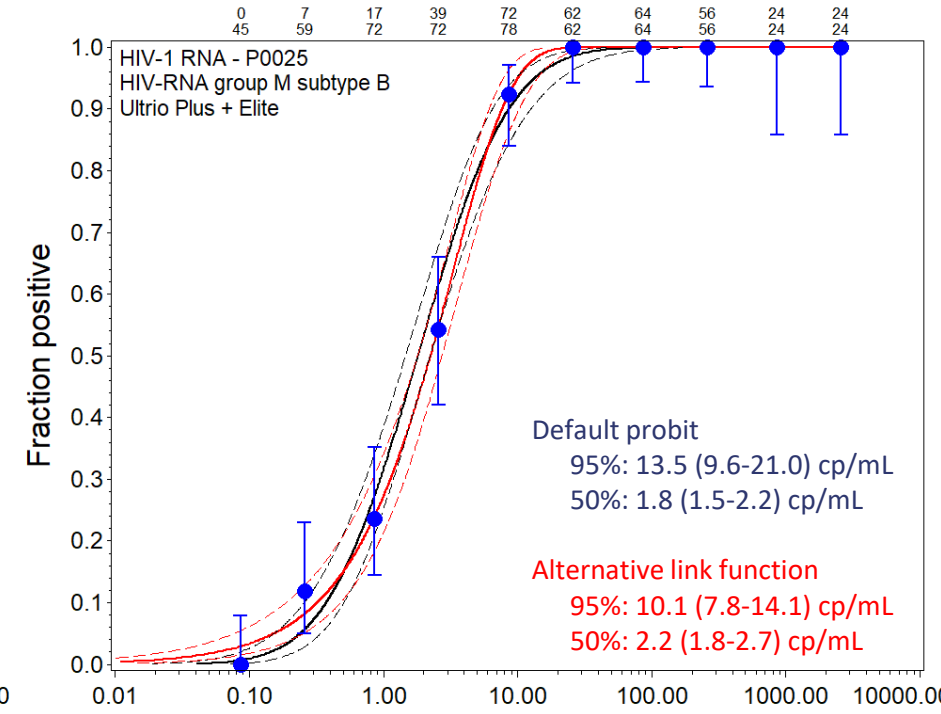
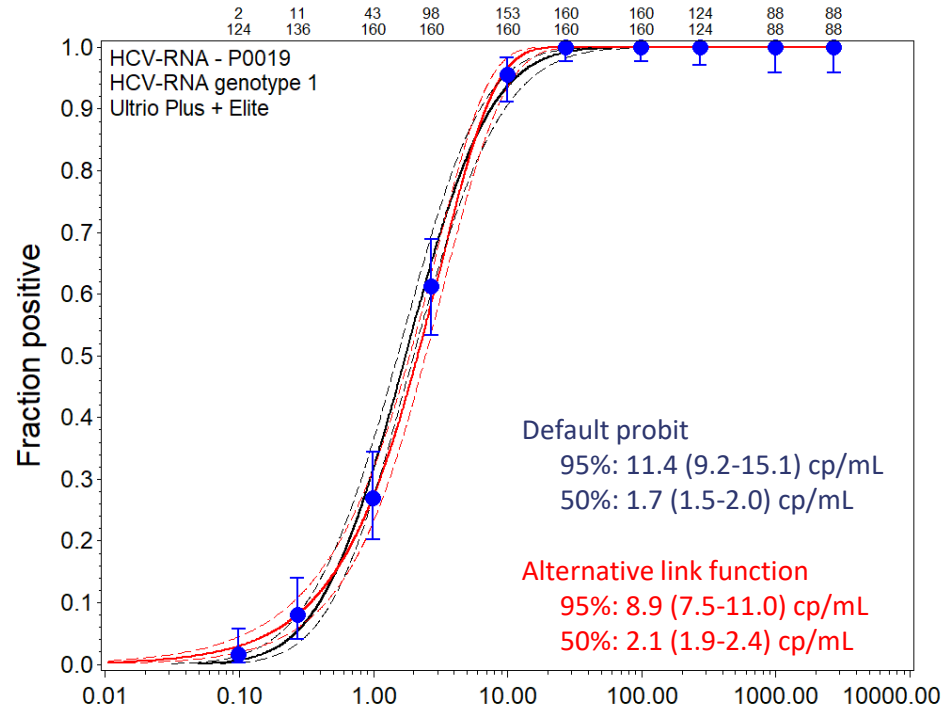
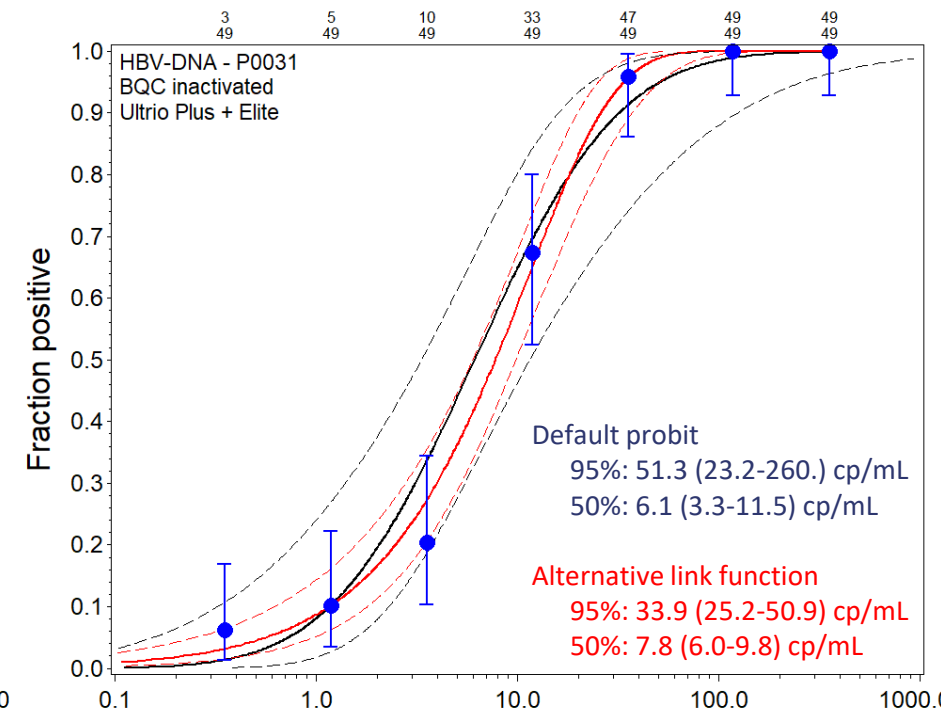
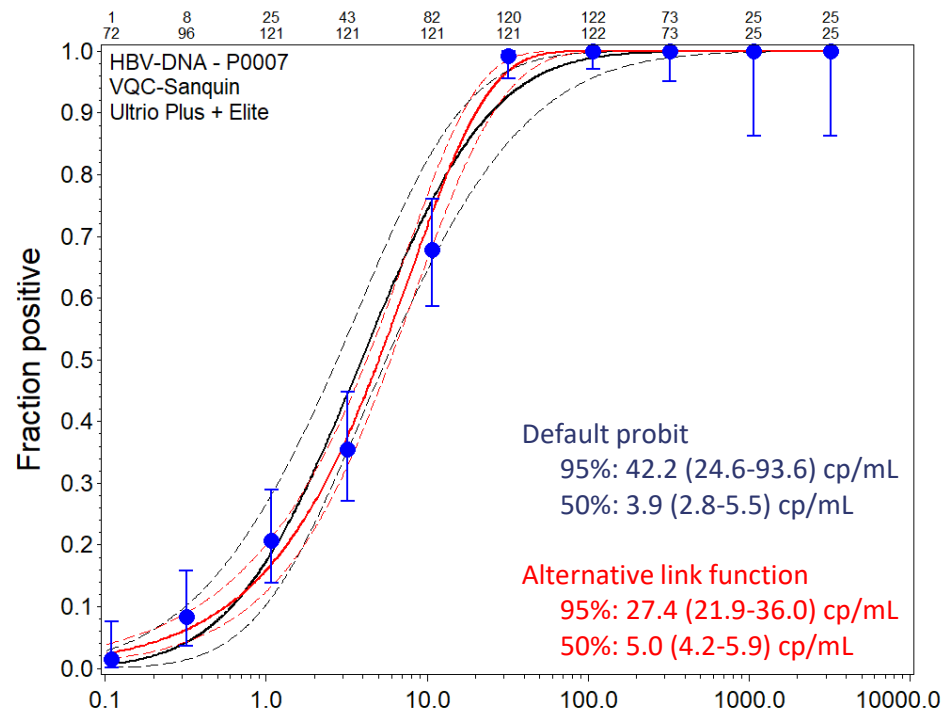
Presumably, the estimates for the 95% and 50% hit rate concentrations are less biased



# Probit analyses default model vs alternative link

More examples with  
same conclusion

Log likelihood statistics  
consistently better



# Conclusion

- Probit analysis is widely used
- Yet it is a fully empirical model
- With large data sets, deviation from perfect symmetry in the curve is readily detectable
- We propose an alternative link function describing the positivity rate as function of the concentration, which is implemented in many standard statistical software packages

Thanks!