LC480 Data Analysis of ISAV Testing at AVC

2011-11-29

Understanding Sample Crossing Points*

- In an amplification reaction, the cycle at which the fluorescence of a sample rises above the background fluorescence is called the “crossing point (Cp)” of the sample.
- A sample’s Cp depends on the initial concentration of nucleic acids in the sample.
- A sample with a lower initial concentration of target nucleic acids requires more amplification cycles to reach the Cp.
- A sample with higher concentration requires fewer cycles. How Cp values are used in a quantification analysis depends on the type of analysis.

*LC480 Operator’s manual, Section 4.2.1.

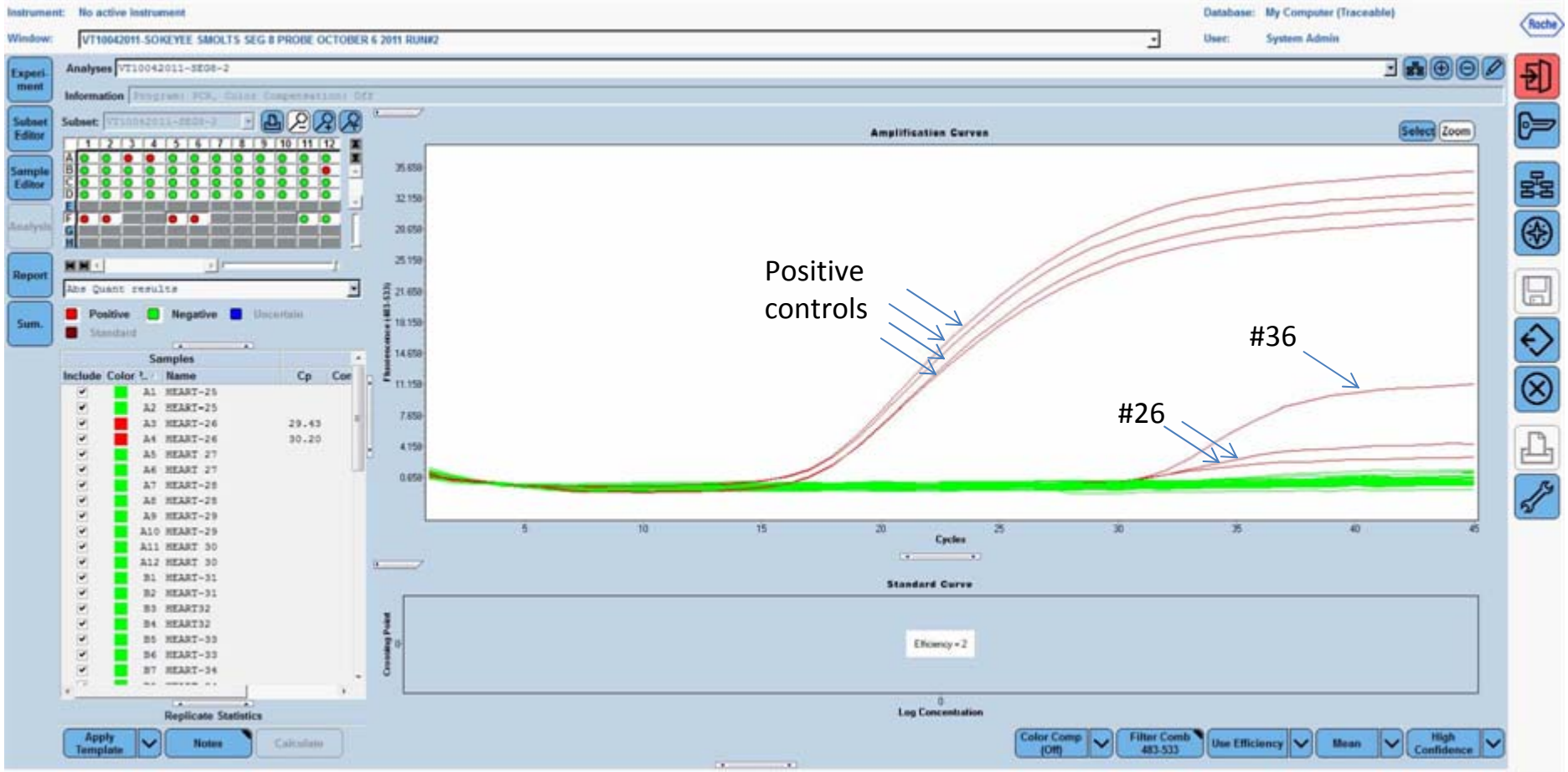
Understanding Sample Crossing Points

- When LC480 assigns a crossing-point to the sample it is interpreted as “reactive”
- Visual inspection of amplification curves is recommended to confirm the true Cp status
- “reactive” can be
  - positive
  - negative
  - inconclusive (LC480 Software also uses the term ”uncertain”)
  - suspicious

Sample Set #1 – 48 Sockeye smolt hearts VT10042011_October 12 2011

Lab #	Sample ID	ISAV seg 8 Probe, Cts Detects all ISAV	ISAV Seg 6 Probe IP* Cts Detects European genotype	ISAV Seg 6 Probe IP* Cts Detects North American genotype
VT 10042011-1	Sokeye heart _1	0	not done	not done
VT 10042011-2	Sokeye heart _2	0	not done	not done
VT 10042011-3	Sokeye heart _3	0	not done	not done
VT 10042011-4	Sokeye heart _4	0	not done	not done
VT 10042011-5	Sokeye heart _5	0	not done	not done
VT 10042011-6	Sokeye heart _6	0	not done	not done
VT 10042011-7	Sokeye heart _7	0	not done	not done
VT 10042011-8	Sokeye heart _8	0	not done	not done
VT 10042011-9	Sokeye heart _9	0	not done	not done
VT 10042011-10	Sokeye heart _10	0	not done	not done
VT 10042011-11	Sokeye heart _11	0	not done	not done
VT 10042011-12	Sokeye heart _12	0	not done	not done
VT 10042011-13	Sokeye heart _13	0	not done	not done
VT 10042011-14	Sokeye heart _14	0	not done	not done
VT 10042011-15	Sokeye heart _15	0	not done	not done
VT 10042011-16	Sokeye heart _16	0	not done	not done
VT 10042011-17	Sokeye heart _17	0	not done	not done
VT 10042011-18	Sokeye heart _18	0	not done	not done
VT 10042011-19	Sokeye heart _19	0	not done	not done
VT 10042011-20	Sokeye heart _20	0	not done	not done
VT 10042011-21	Sokeye heart _21	0	not done	not done
VT 10042011-22	Sokeye heart _22	0	not done	not done
VT 10042011-23	Sokeye heart _23	0	not done	not done
VT 10042011-24	Sokeye heart _24	0	not done	not done
VT 10042011-25	Sokeye heart _25	0	not done	not done
VT 10042011-26	Sokeye heart _26	29.82	32.7	0
VT 10042011-27	Sokeye heart _27	0	not done	not done
VT 10042011-28	Sokeye heart _28	0	not done	not done
VT 10042011-29	Sokeye heart _29	0	not done	not done
VT 10042011-30	Sokeye heart _30	0	not done	not done
VT 10042011-31	Sokeye heart _31	0	not done	not done
VT 10042011-32	Sokeye heart _32	0	not done	not done
VT 10042011-33	Sokeye heart _33	0	not done	not done
VT 10042011-34	Sokeye heart _34	0	not done	not done
VT 10042011-35	Sokeye heart _35	0	not done	not done
VT 10042011-36	Sokeye heart _36	30.86	33.21	0
VT 10042011-37	Sokeye heart _37	0	not done	not done
VT 10042011-38	Sokeye heart _38	0	not done	not done
VT 10042011-39	Sokeye heart _39	0	not done	not done
VT 10042011-40	Sokeye heart _40	0	not done	not done
VT 10042011-41	Sokeye heart _41	0	not done	not done
VT 10042011-42	Sokeye heart _42	0	not done	not done
VT 10042011-43	Sokeye heart _43	0	not done	not done
VT 10042011-44	Sokeye heart _44	0	not done	not done
VT 10042011-45	Sokeye heart _45	0	not done	not done
VT 10042011-46	Sokeye heart _46	0	not done	not done
VT 10042011-47	Sokeye heart _47	0	not done	not done
VT 10042011-48	Sokeye heart _48	0	not done	not done
ADL-ISAV (European genotype)		17.24	18.5	0
NBISAV01 (North American genotype)		17.17	0	15.1
NTC (water)		0	0	0
SIMON FRASER UNIVERSTY SAMPLES SUBMITTED BY NICOLE GERBRANT: SHIPPED ON ICE PACK, RECIEVED IN GOOD CONDITION WITH ICE PACKS STIL				

Sample Set #1 - screening test – segment 8 – Oct 6



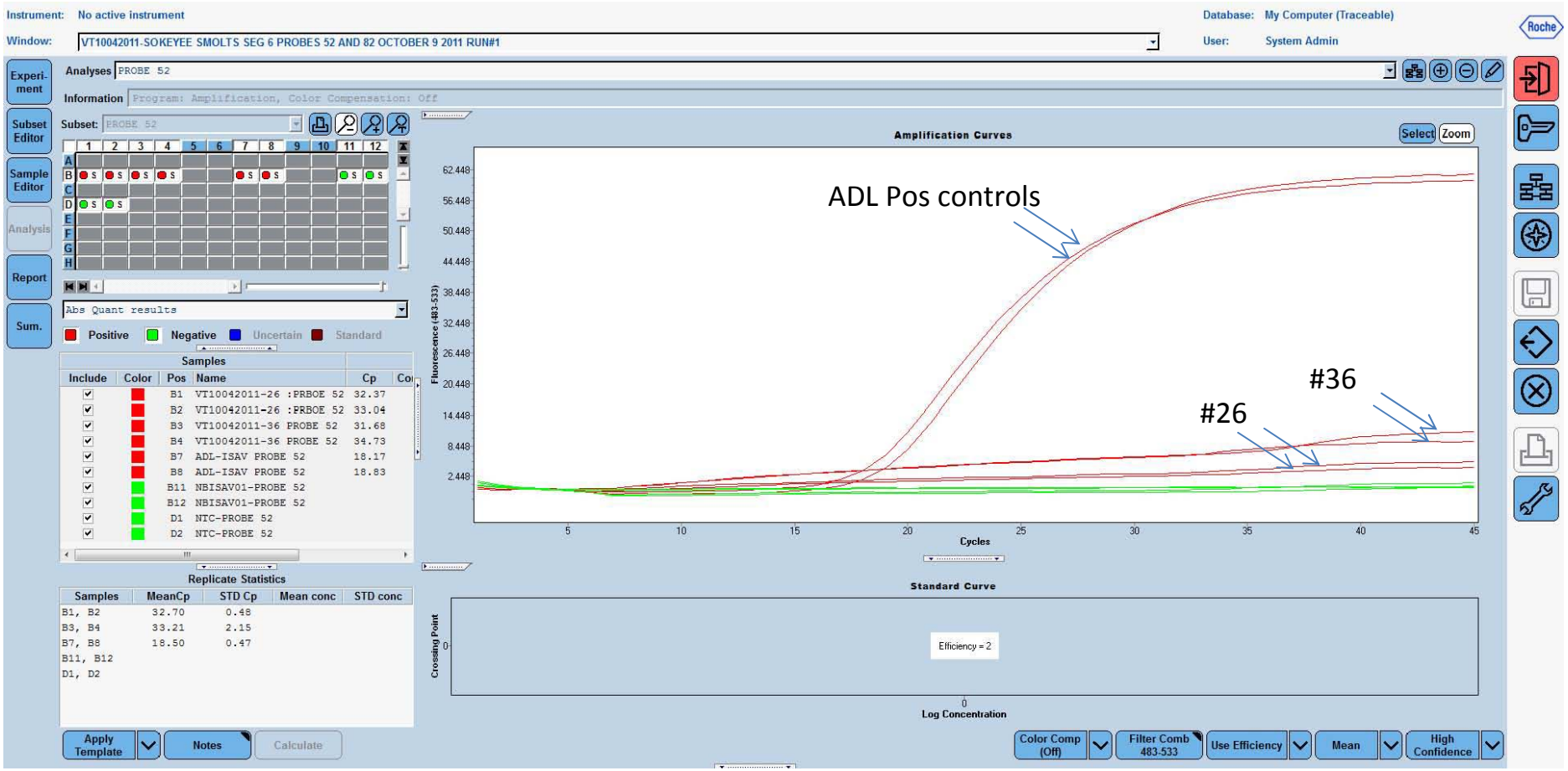
Controls - tested in duplicates

- Two positive controls (European and North American genotypes), each tested in duplicate– all positive controls were positive as expected.
- NTC (no template control) - NTC was negative in duplicate.

Samples - tested in duplicates

- 46 samples reported negative
- Sample #26 reported positive, mean Cp 29.82 - a weak positive in my opinion - *my recommendation would have been to re-test.*
- Sample #36 reported positive, mean Cp 30.86 - *sample #36 was positive only once , report didn't indicate 1 of 2 was negative.*

Sample Set #1 - probe 52 genotyping test – segment 6



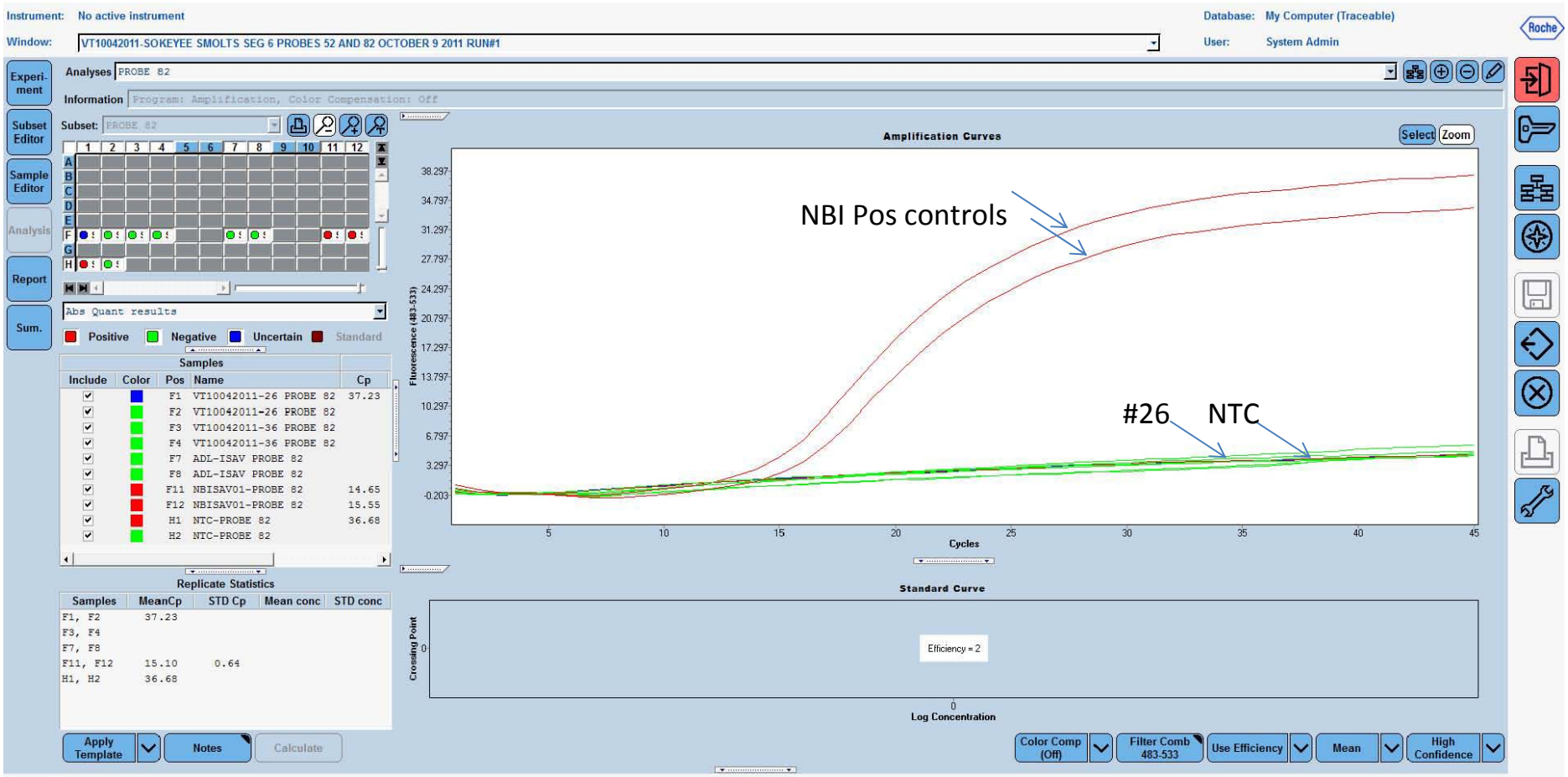
Controls - tested in duplicates

- Two positive controls (European and North American genotypes), each tested in duplicate–positive control for the European genotype (ADL) was positive in duplicate as expected and positive control for the North-American genotype (NBI) was negative in duplicate as expected.
- NTC (no template control) - NTC was negative in duplicate.

Samples - tested in duplicates

- #26 reported positive in duplicate , mean Cp 32.7 - *the shape of curves is too "flat" and not indicative of true positives - recommendation would have been to re-test*
- #36 reported positive in duplicate , mean Cp 33.21 - *the shape of curves is too "flat" and not indicative of true positives - recommendation would have been to re-test*

Sample Set #1 - probe 82 genotyping test – segment 6



Controls - tested in duplicates

- Two positive controls (European and North American genotypes), each tested in duplicate–positive control for the European genotype (ADL) was negative in duplicate as expected and positive control for the North-American genotype (NBI) was positive in duplicate as expected.
- NTC (no template control) – reported negative – *however, the software called one NTC well negative, one "reactive" Cp 36.68- well H1 – not reported – when NTC reacts a test is typically considered invalid. My interpretation of NTC "reactivity" would be negative, but software called it as call based on 2nd Derivative Maximum method.*

Samples- tested in duplicates

- #26 reported negative - *1 of 2 #26 was "uncertain" with Cp 37.23 – not reported – call based on 2nd Derivative Maximum method - my interpretation negative.*
- #36 negative *as expected.*

Sample Set #2- 20 Sample - Sockeye, Chinook, & Coho Hearts and Gills_VT10142011_October 20 2011

Lab #	Sample ID	ISAV seg 8 Probe, Cts Detects all ISAV	ISAV Seg 6 Probe 52 Cts Detects European genotype	ISAV Seg 6 Probe 82 Cts Detects North American genotype
VT 10142011-49	Coho heart-1	0	not done	not done
VT 10142011-50	Chinook heart-2	0	not done	not done
VT 10142011-51	Coho heart-3	0	not done	not done
VT 10142011-52	Chinook heart-4	0	not done	not done
VT 10142011-53	Coho heart-6	0	not done	not done
VT 10142011-54	Sokeye heart-7	0	not done	not done
VT 10142011-55	Coho heart-8	0	not done	not done
VT 10142011-56	Coho heart-9	0	not done	not done
VT 10142011-57	Chum heart-10	0	not done	not done
VT 10142011-58	Coho heart-11	33.61 (1/2)	33.06	0
VT 10142011-59	Coho Gill-1	0	not done	not done
VT 10142011-60	Chinook Gill-2	32.99	0	0
VT 10142011-61	Coho Gill-3	0	not done	not done
VT 10142011-62	Chinook Gill-4	0	not done	not done
VT 10142011-63	Coho Gill-6	0	not done	not done
VT 10142011-64	Sokeye Gill-7	0	not done	not done
VT 10142011-65	coho Gill-8	0	not done	not done
VT 10142011-66	Coho Gill-9	0	not done	not done
VT 10142011-67	Chum Gill-10	33.77 (1/2)	0	0
VT 10142011-68	Coho Gill-11	0	not done	not done
ADL-ISAV (European genotype)		16.85	18.45	0
NBISAV01 (North American genotype)		16.43	0	14.73
NTC (water)		0	0	0

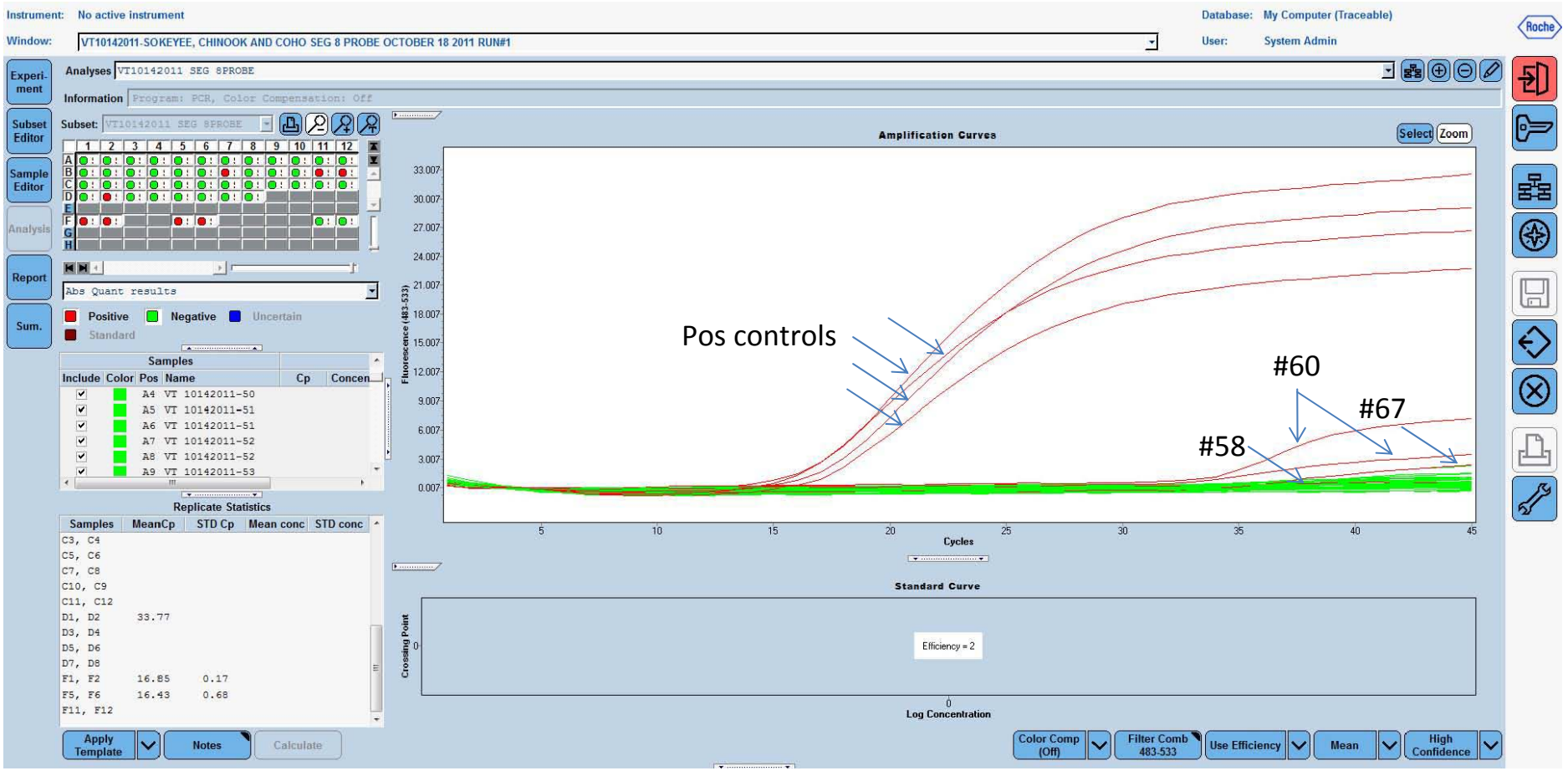
EXPLANATORY NOTES:

1. All samples were tested for ISAV using real-time RT-PCR with TaqMan probe for ISAV segment 8. The result is the number of PCR cycles to reach reliable detection of product (cycle threshold or Ct).
2. Based on this PCR test, 3 of the tissue samples provided to the laboratory were RT-PCR positive. This test means only that 3 tissues contained ISAV sequences of genome segment 8.
3. The 3 tissues that tested positive were further tested using real-time RT-PCR with TaqMan probes for ISAV segment 6 for genotyping.
4. 1 of the 3 tissues tested positive for ISAV of the European genotype. This test did not detect any segment 6 sequences in the other 2 samples.
5. The ISAV sequences detected from the samples could be from viable or non-viable virus. The virus could be pathogenic or non-pathogenic.
6. The presence of ISAV sequences in the tissue samples does not imply that the subject fish had ISA or that ISA is present in the area where the subject fish were collected from.
7. Confirmation of ISAV infection requires virus isolation in cell culture and identification. This additional testing is underway, and results will not be known for another 6 weeks.
8. The laboratory did not participate in the collection of the samples or in the custody of the samples prior to receipt of the samples. The laboratory therefore cannot guarantee the integrity of the samples.
9. For convenience, the samples are identified using the labels provided by the party who requested testing by the laboratory.
10. The samples were tested as received at the laboratory.
11. In accordance with the Health of Animals Act, the test results have been reported to representatives from the CFIA by the laboratory.

INTERPRETATION:

1. Ct up to 40 are positive. Ct between 40.1 and 45 are considered suspicious. Sample is negative if there is no Ct value.

Sample Set #2- screening test – segment 8



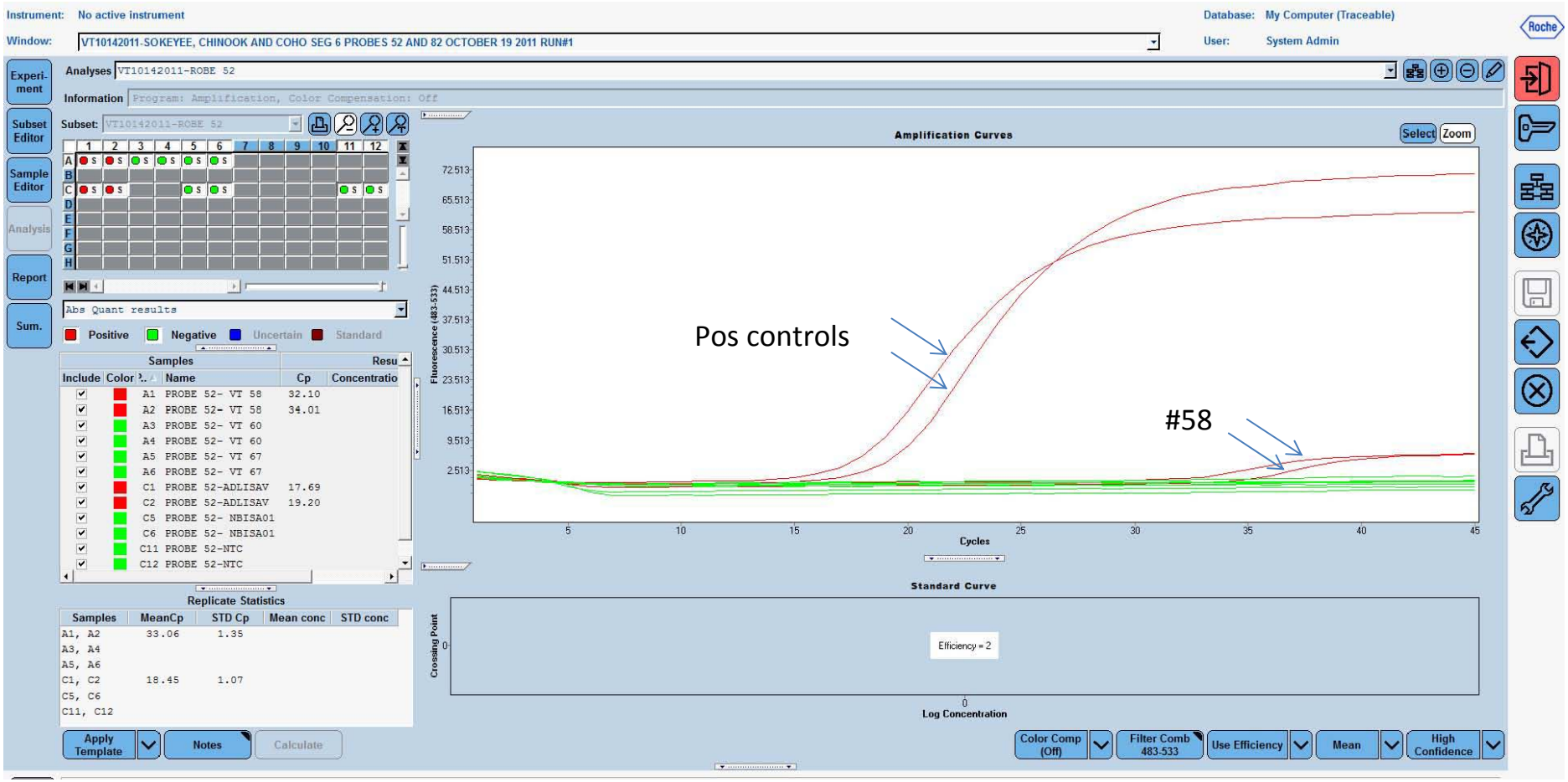
Controls - tested in duplicates

- Two positive controls (European and North American genotypes), each tested in duplicate– all positive controls were positive as expected.
- NTC (no template control) - NTC was negative in duplicate.

Samples - tested in duplicates

- 17 samples reported as negative
- #58, one of two reported positive, Cp 33.61 – *my interpretation - negative*
- #60, two of two reported positive, mean Cp 32.99 - *in my opinion one was weak positive, almost inconclusive, one positive*
- #67, one of two reported positive, Cp 33.77, - *inconclusive in my opinion - recommendation would have been to re-test.*

Sample Set #2- Genotyping Run 1 – probe 52 segment 6



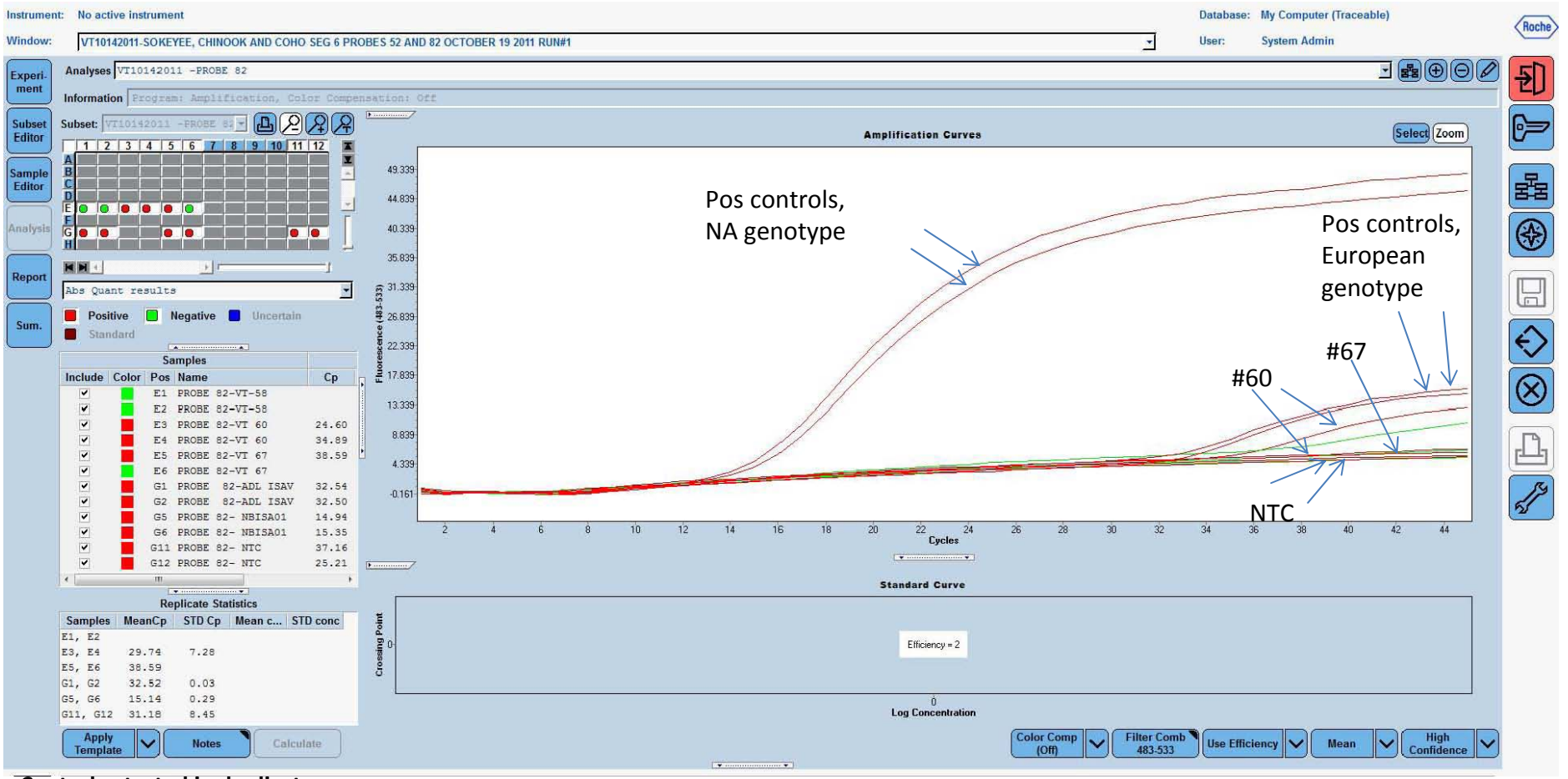
Controls - tested in duplicates

- Two positive controls (European and North American genotypes), each tested in duplicate–positive control for the European genotype (ADL) was positive in duplicate as expected and positive control for the North-American genotype (NBI) was negative in duplicate as expected.
- NTC (no template control) - NTC was negative in duplicate.

Samples - tested in duplicates

- #58, reported positive, Cp 33.06 - *weak reactors - retest*
- #60, reported negative.
- #67, reported negative.

Sample Set #2- Genotyping Run 1 – probe 82 segment 6



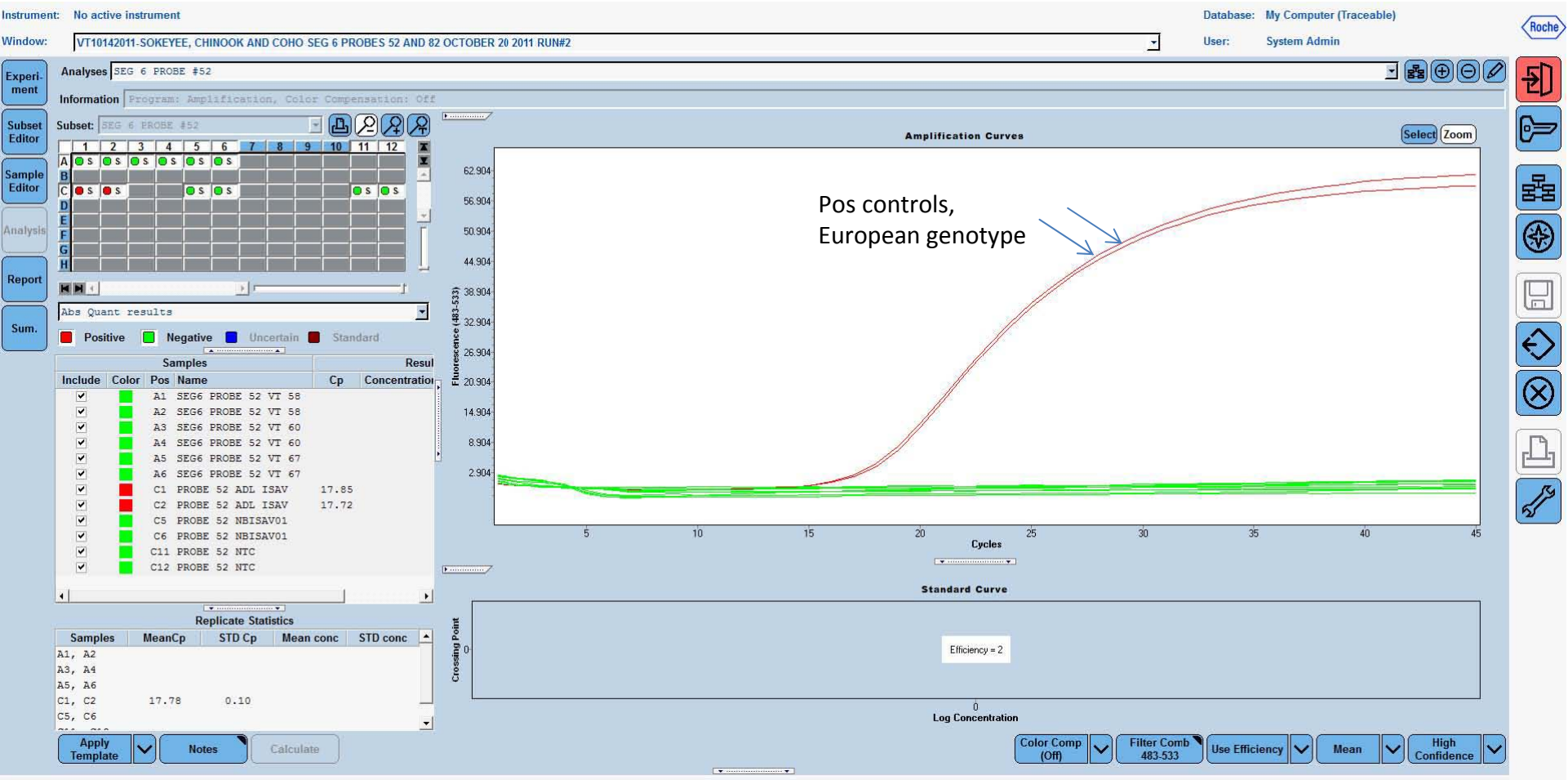
Controls - tested in duplicates

- Two positive controls (European and North American genotypes) were each tested in duplicate. Positive control for the European genotype (ADL) was positive in duplicate *(not expected)* and positive control for the North-American genotype (NBI) was positive in duplicate as expected.
- NTC (no template control) – reported negative – *however, the software called reactive in duplicate, Cp 37.16 and 25.21– not reported – when NTC reacts a test is typically considered invalid. My interpretation of NTC "reactivity" would be negative, but software called it as call based on 2nd Derivative Maximum method.*

Samples- tested in duplicates

- #58 – negative
- #60 - reactive in duplicate (Cp 24.60 and 34.89) - *My interpretation of "reactivity" would be "negative", a software artefact, not reported?*
- #67 - 1 of 2 reactive (Cp 38.59) - *My interpretation of "reactivity" would be "negative", a software artefact, not reported?*

Sample Set #2- Genotyping Run 2 – probe 52 segment 6



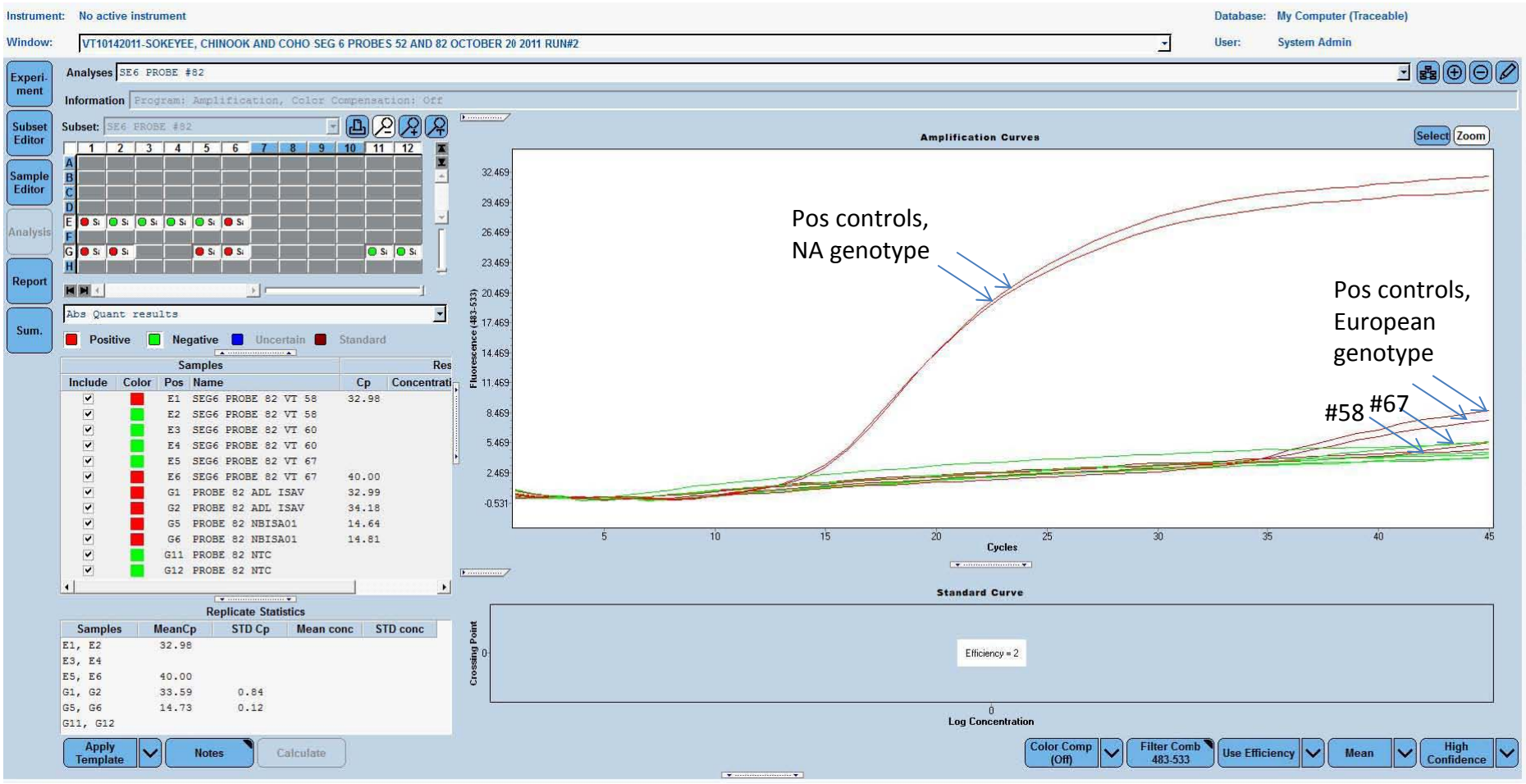
Controls - tested in duplicates

- Two positive controls (European and North American genotypes) were each tested in duplicate. Positive control for the European genotype (ADL) was positive in duplicate as expected and positive control for the North-American genotype (NBI) was negative in duplicate as expected.
- NTC (no template control) - NTC was negative in duplicate.

Samples - tested in duplicates

-All samples were negative for European genotype in run 2 – sample #58 was reported positive based on the first run

Sample Set #2- Genotyping Run 2 – probe 82 segment 6



Controls - tested in duplicates

- Two positive controls (European and North American genotypes) were each tested in duplicate. Positive control for the European genotype (ADL) was positive in duplicate *(not expected)* and positive control for the North-American genotype (NBI) was positive in duplicate as expected.
- NTC (no template control) –negative

Samples- tested in duplicates

- All samples were reported negative - *software assigned CPs to sample #58 (1 of 2) and sample 67 (1 of 2). In my opinion these samples are negative, but this was not reported.*

Conclusions

- 2nd Derivative Maximum method used for calls to classify samples as pos/neg/sus
  - Advantage – minimal user input
  - Disadvantage – can generate false reactors and doesn't allow baseline adjustments
- Indiscriminate use of the 2nd Derivative Maximum method for data analysis responsible for most errors
- Reporting contradictions for control aberrations noticed
- AVC report validity compromised