



*building towards*

**canadian Immunohistochemistry Quality control**

**cIQc**

## Run 60 B-Raf v600e

Sept 2016

The purpose of the challenge is to use image analysis to provide technical evaluation of IHC protocols. The graphs below show the level of optical intensity of staining, providing an objective analysis of laboratory performance which can be considered when evaluating clinical samples.

- The challenge consisted of a 12-core cell line tissue microarray. Cell lines are courtesy of Horizon (HDx™) with BRAF V600E expression having been characterized extensively in these cell lines.
- Labs were asked to perform BRAF V600E immunohistochemical staining according to their usual laboratory protocol.
- Results were entered in TMA scorer <http://www.tmscorersystem.ca/login.php>
- Slides were scanned and Image Analysis was performed by Visiopharm

While this run is a step forward in our efforts to have automated and more objective assessment of proficiency testing runs, this approach is not yet ready for routine use, and although the results are of interest, they are not, in our opinion, actionable, as both the substrate (cell blocks of cell lines) and the image analysis interpretation are not sufficiently validated yet for clinical use.

### BRAF V600E scoring

**S** - Strong – indicates a V600E mutation is present. Weak, moderate, or strong cytoplasmic positivity in cells.

**M** - Moderate cytoplasmic positivity

**W** – Weak cytoplasmic positivity

**N** - Negative – indicates no mutation present. No staining in cells.

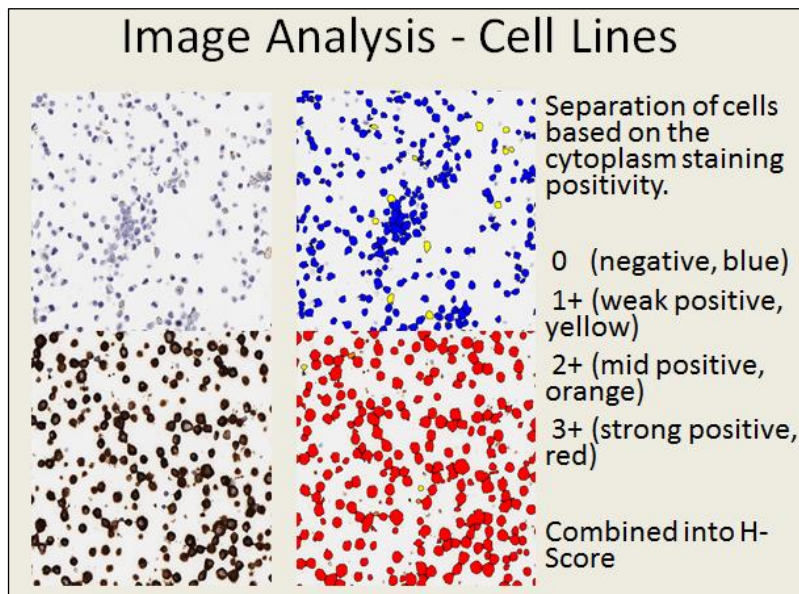
**U** - Core unsatisfactory for analysis (e.g. technical issues, missing core, etc.)

## Self-Assessment Results - B-Raf v600e

Labs / Cores	101	114	116	160	175	189	191	193	202	215	217	228	reference
1	W	U	N	N	N	N	N	N	N	S	N	W	N
2	S	S	M	S	M	M	M	W	M	S	M	M	M
3	M	M	W	M	W	W	M	W	M	M	W	W	W
4	S	S	S	S	S	S	S	S	S	S	S	S	S
5	W	U	N	N	N	N	N	N	N	M	N	W	N
6	M	S	W	M	M	W	W	W	M	S	M	M	M
7	M	S	W	W	W	W	W	W	M	S	W	M	W
8	S	S	S	S	S	S	S	S	S	S	S	S	S
9	W	U	N	N	N	N	N	N	N	S	N	N	N
10	M	S	M	M	M	W	M	W	M	M	M	M	M
11	M	S	W	W	W	W	W	W	W	M	M	W	W
12	S	S	S	S	S	M	S	S	S	S	S	S	S

### Image Analysis

Image analysis was performed on the slides returned to our office. Cell lines ( from Horizon) were analyzed using ONCOTopix and BRAF APP courtesy of Visiopharm.

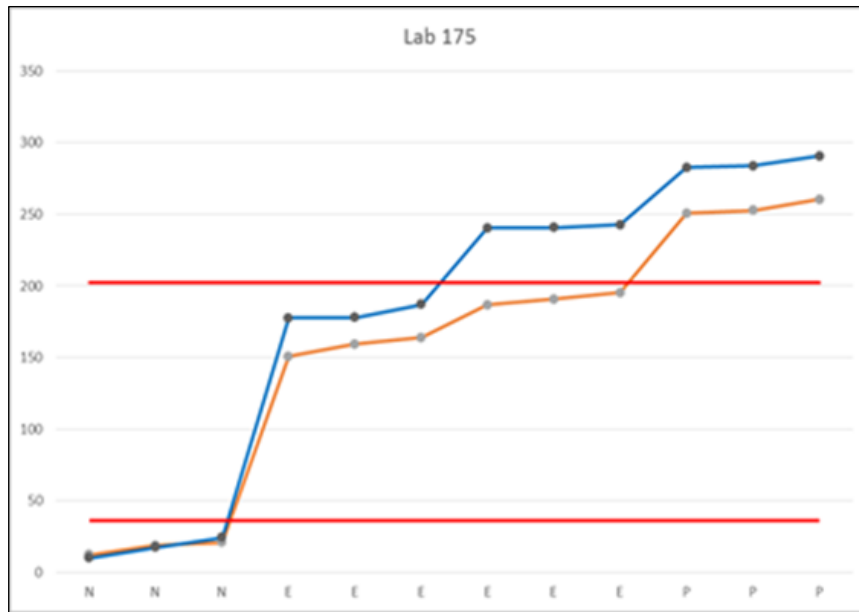


H-Score development from lowest reacting core to highest (Lab profile)

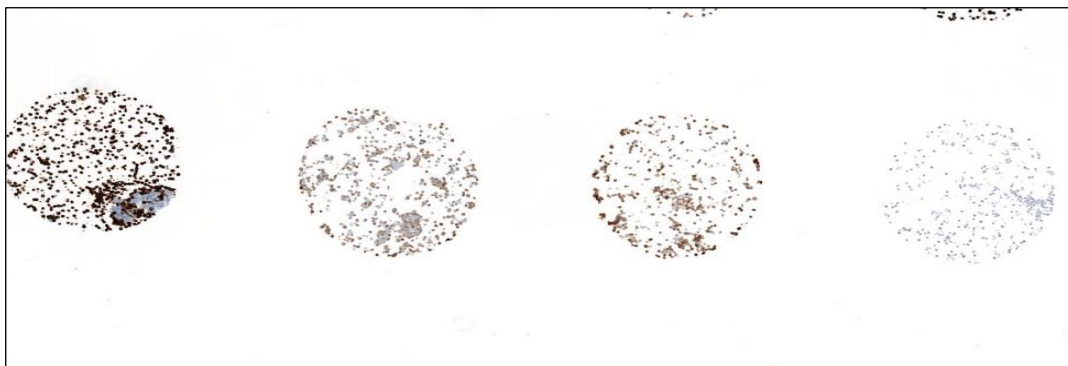
- Individual lab results were compared to the expected output (Reference Lab)
- The cut-offs were calculated for positive intermediate and negative cores

## Image Analysis Graphical Interpretation

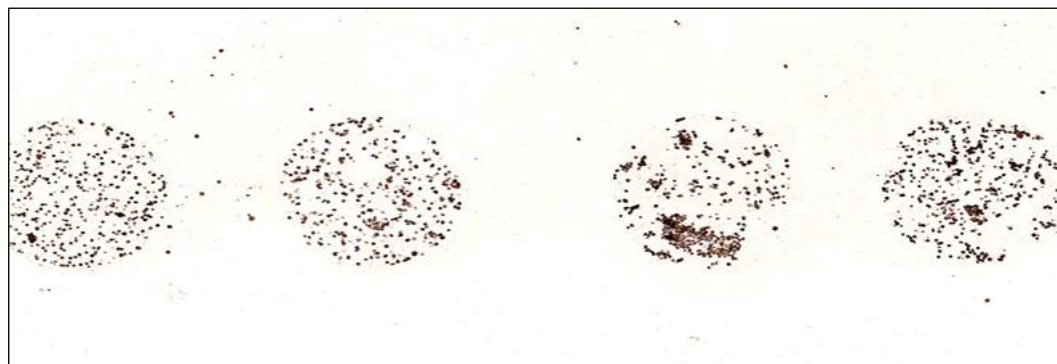
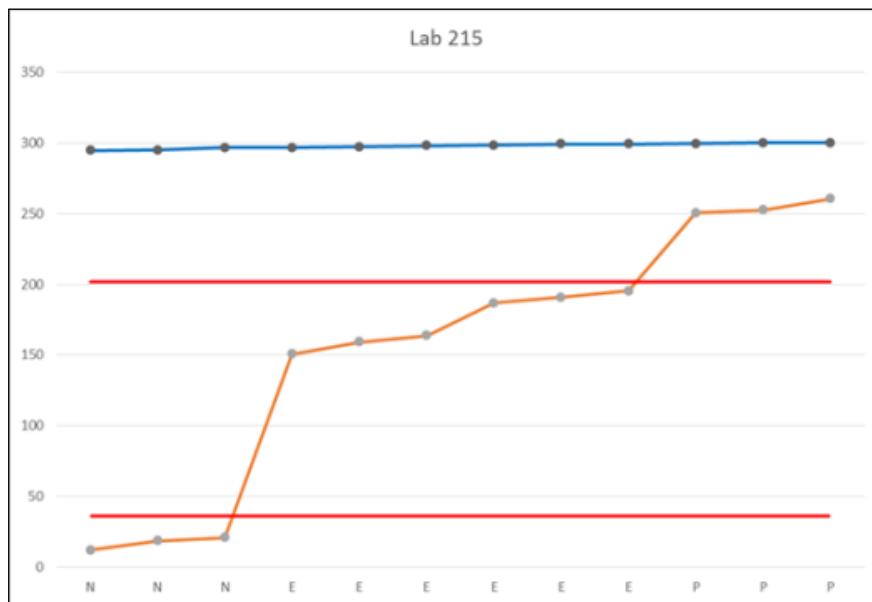
A clear dynamic range of negative, weak intermediate, moderate intermediate and strong staining, of the Run 60 slide, results in four corresponding plateaus when the H-Scores are plotted. This can be seen in the example below from Lab 175 (blue plot line). Orange plot line is the reference lab.



The range of staining of strong, intermediate, weak intermediate and negative (lab 175) can be seen microscopically in the image below.



Lab 215 (blue line) shows no dynamic range which is reflected in the image analysis results and can be visualised in the micrographs below. The orange plot is the reference lab.

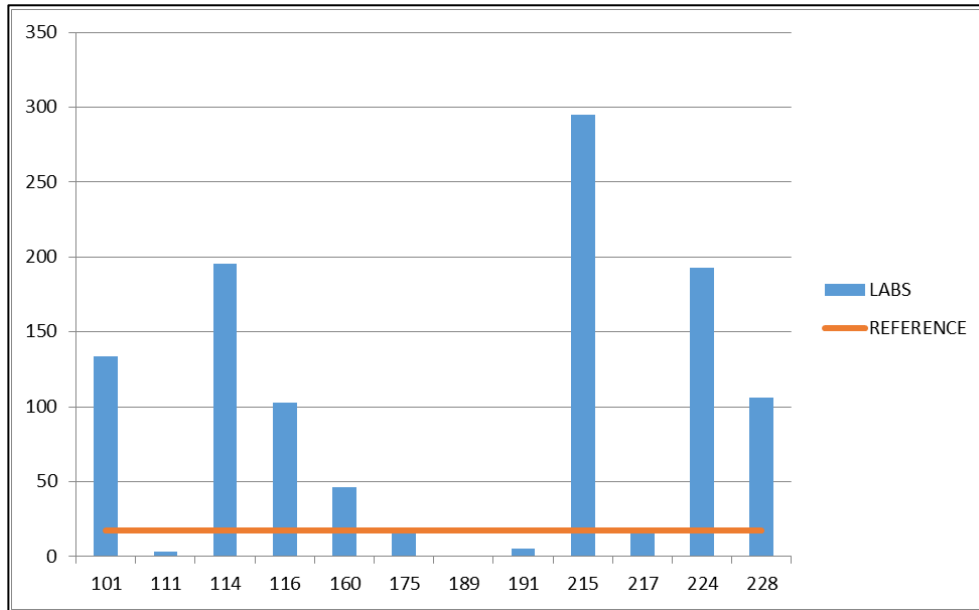


Lab 215

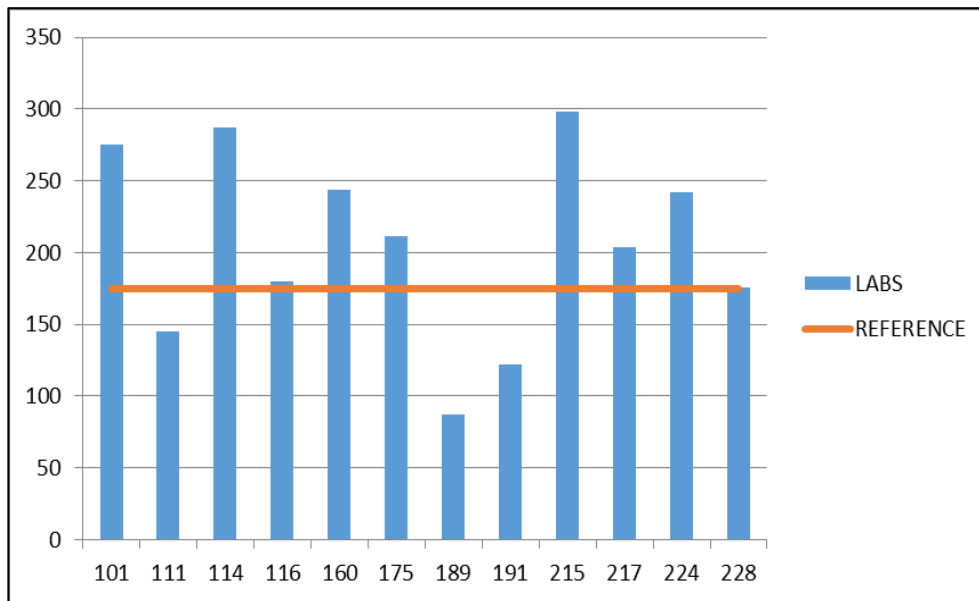
## Combined Results

Run 60 results for each lab were plotted by average core intensity (H-Score) for strong, intermediate (weak and moderate) and negative expressing cores. The orange line is the average H-Score of the reference laboratory.

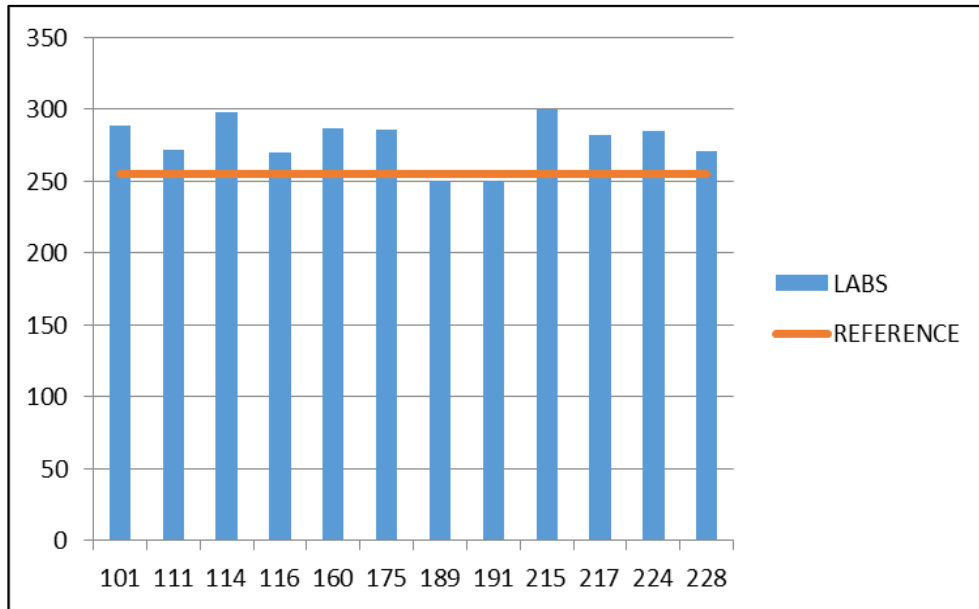
### Negative Expression Cores



### Intermediate Expressing Cores



## Strong Expressing Cores



## Individual Laboratory Image Analysis Results

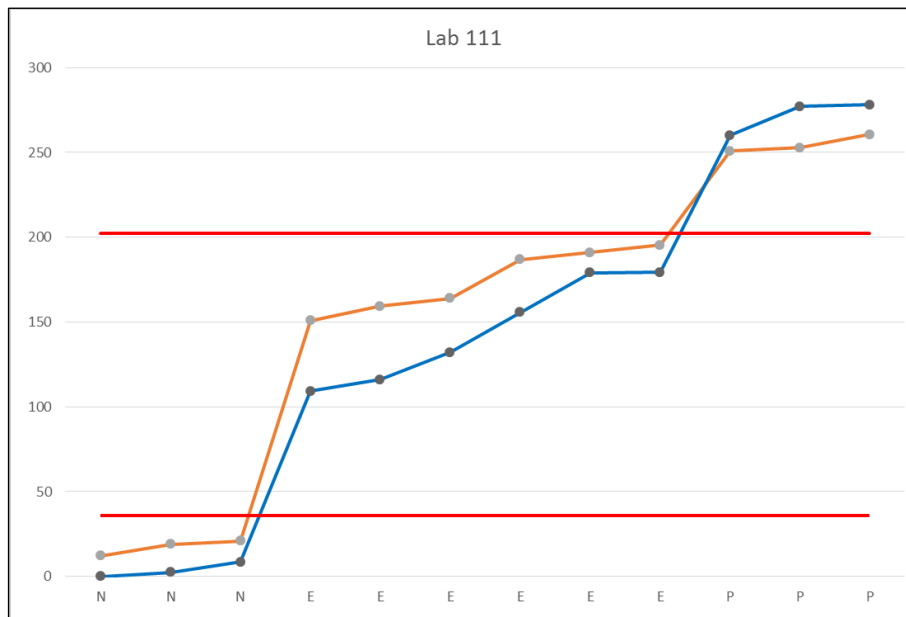
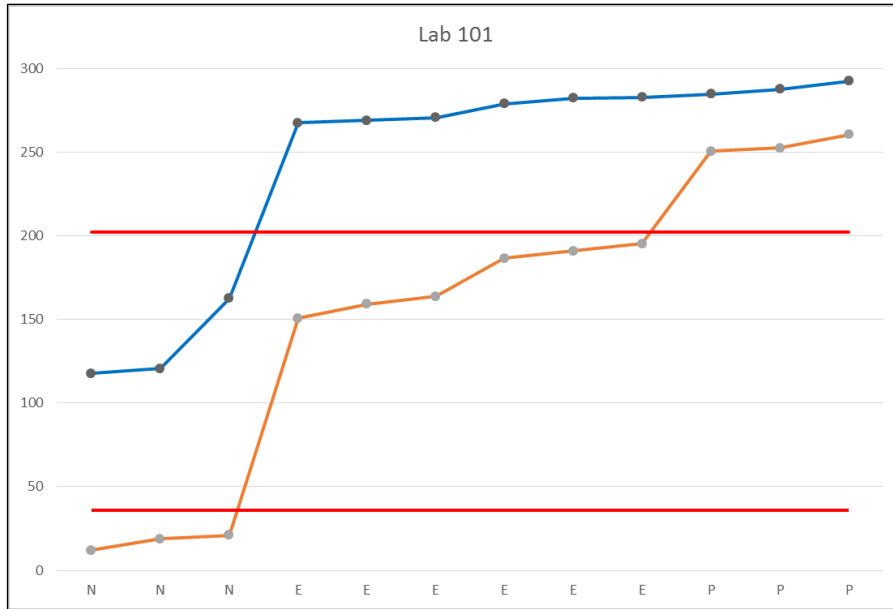
X axis = H-Score.  orange plot = reference results

The H-Scores of individual labs were plotted and graphed with the reference lab and reference lines indicating the expected range of staining intensity for negative, intermediate and strong expressing cores.



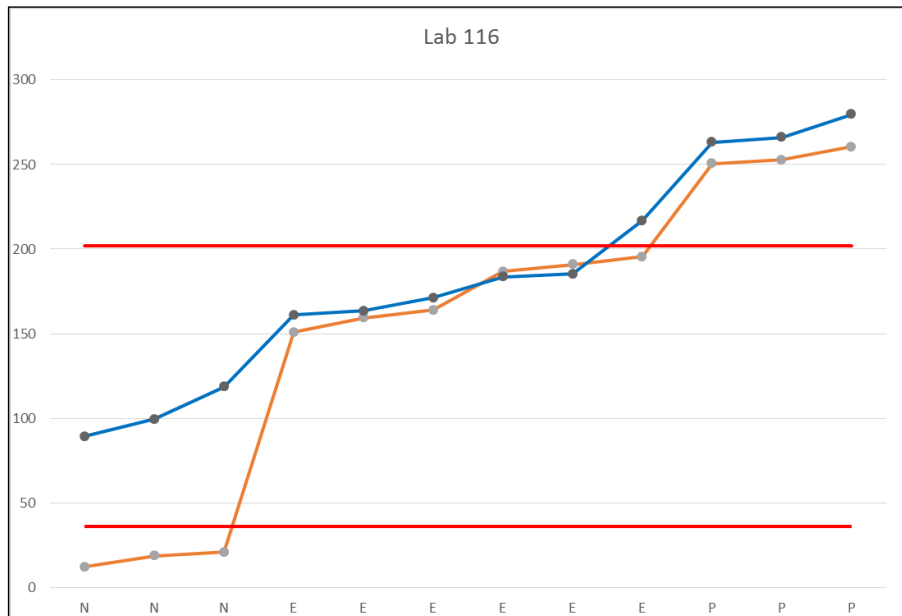
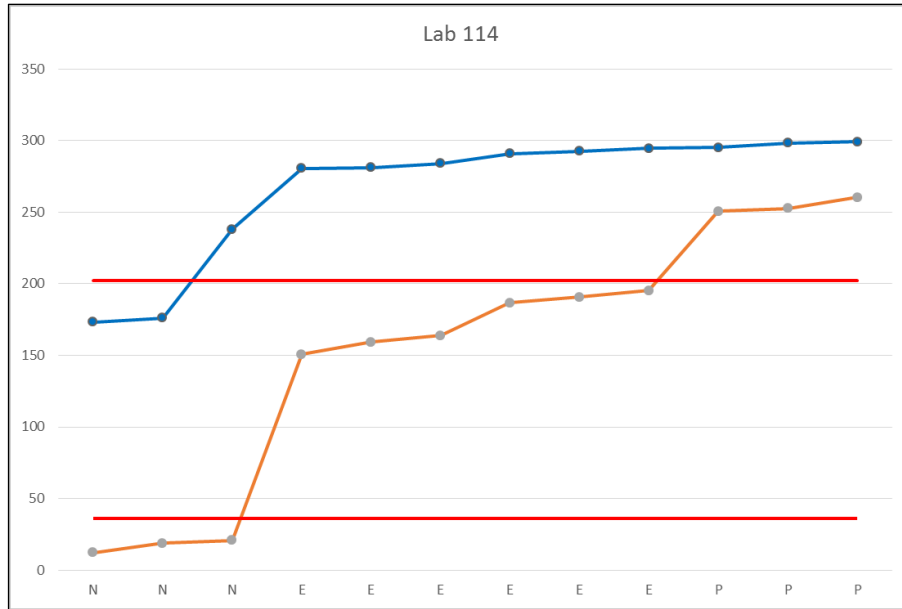
# Individual Laboratory Image Analysis Results

X axis = H-Score. \_\_\_\_\_ Blue plot = lab results, \_\_\_\_\_ orange plot = reference lab results



# Individual Laboratory Image Analysis Results

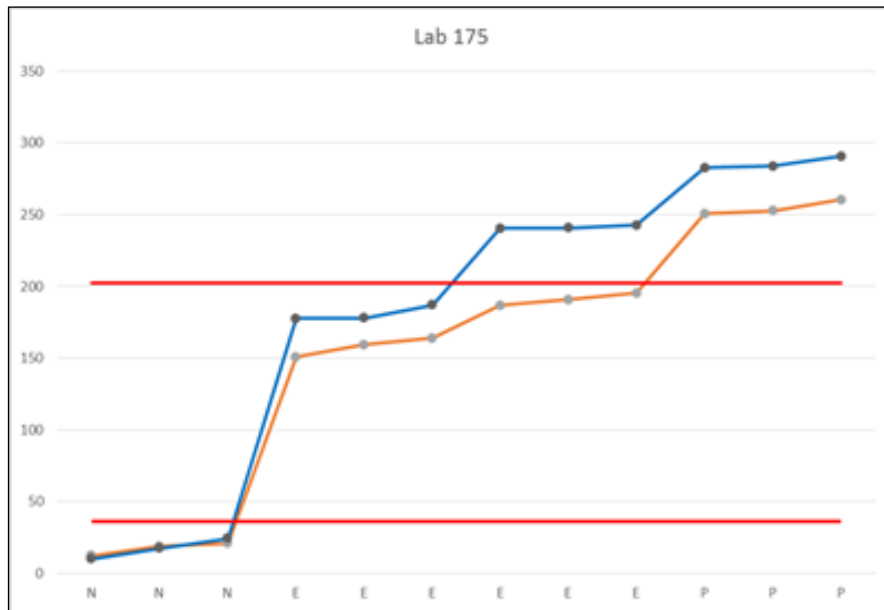
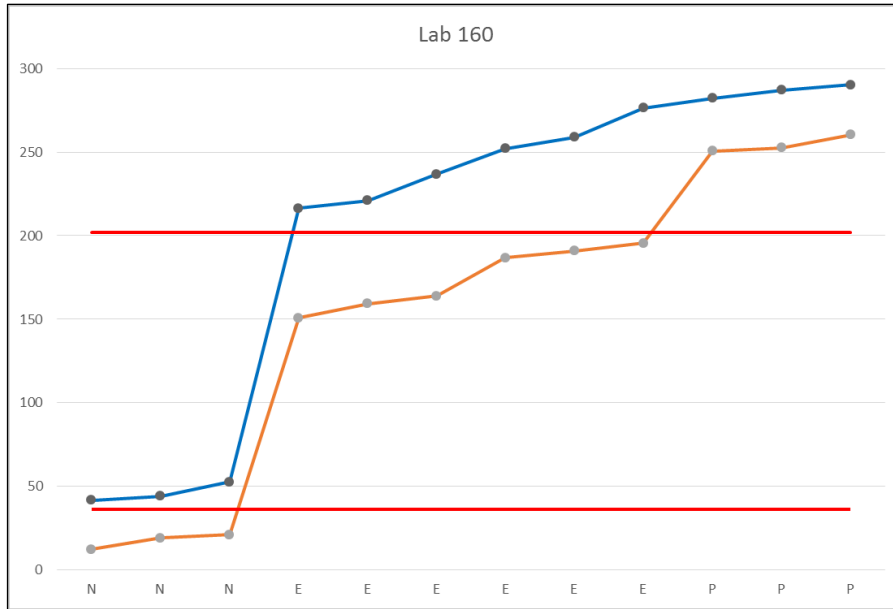
X axis = H-Score. Blue plot = lab results, orange plot = reference lab results





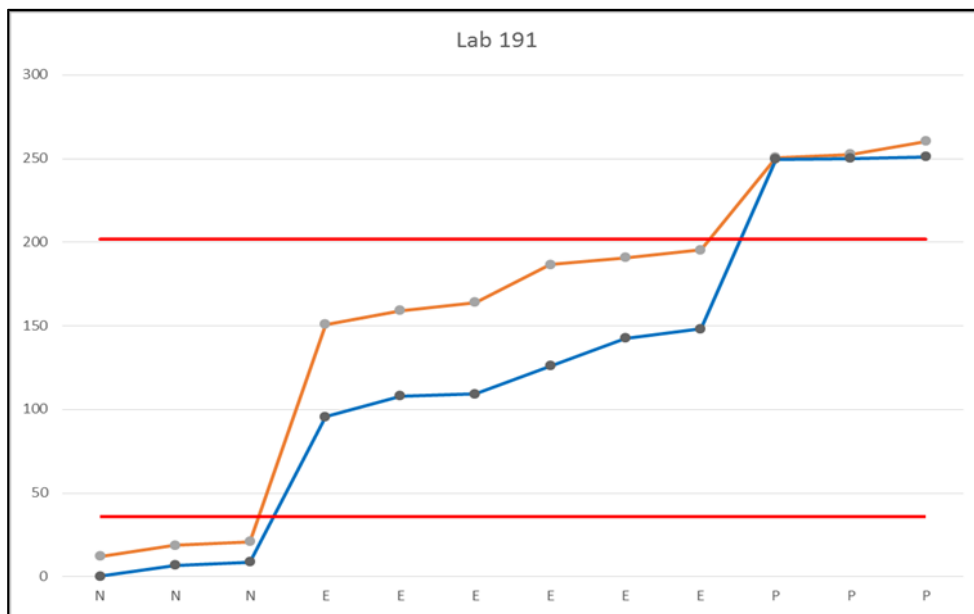
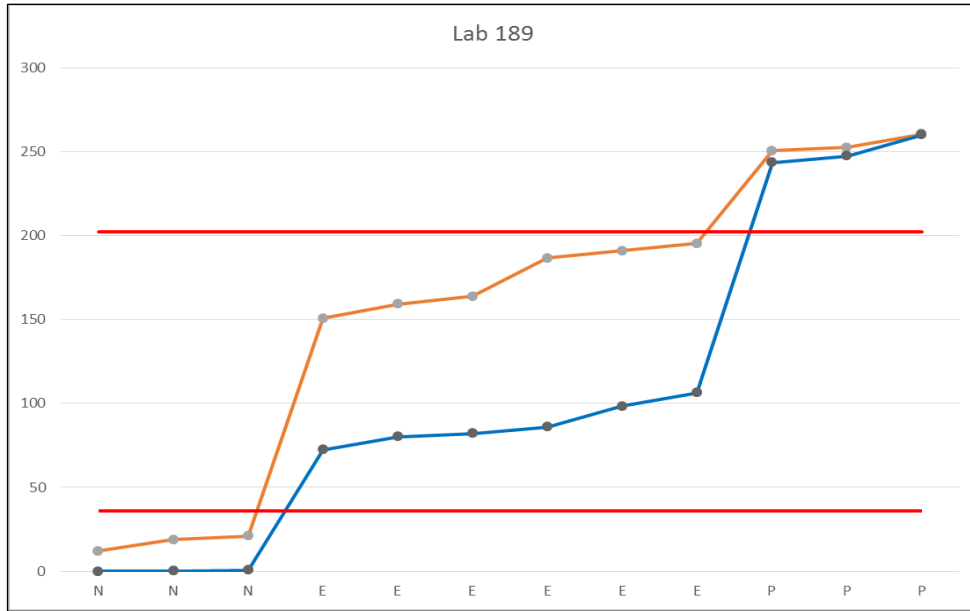
# Individual Laboratory Image Analysis Results

X axis = H-Score. Blue plot = lab results, orange plot = reference lab results



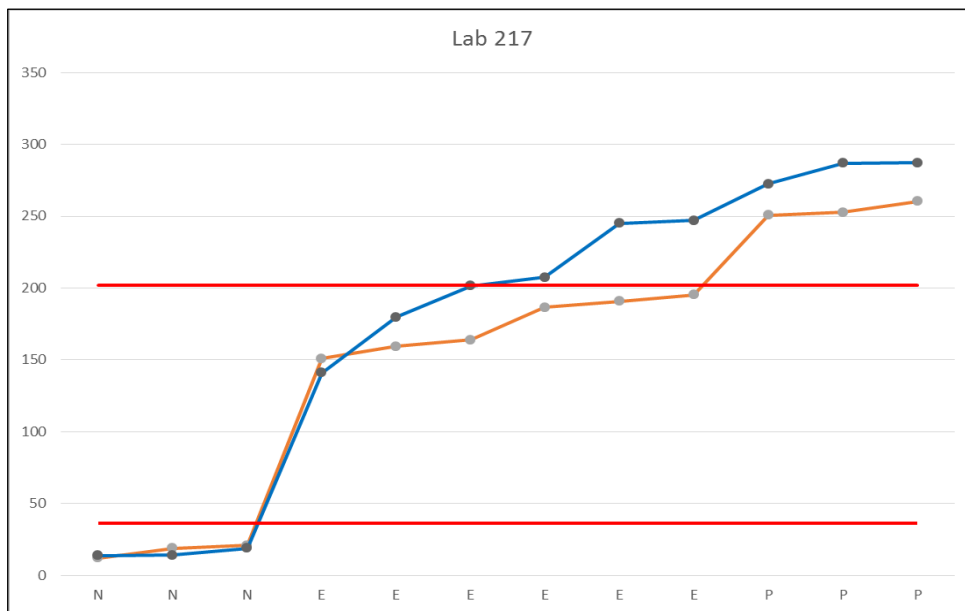
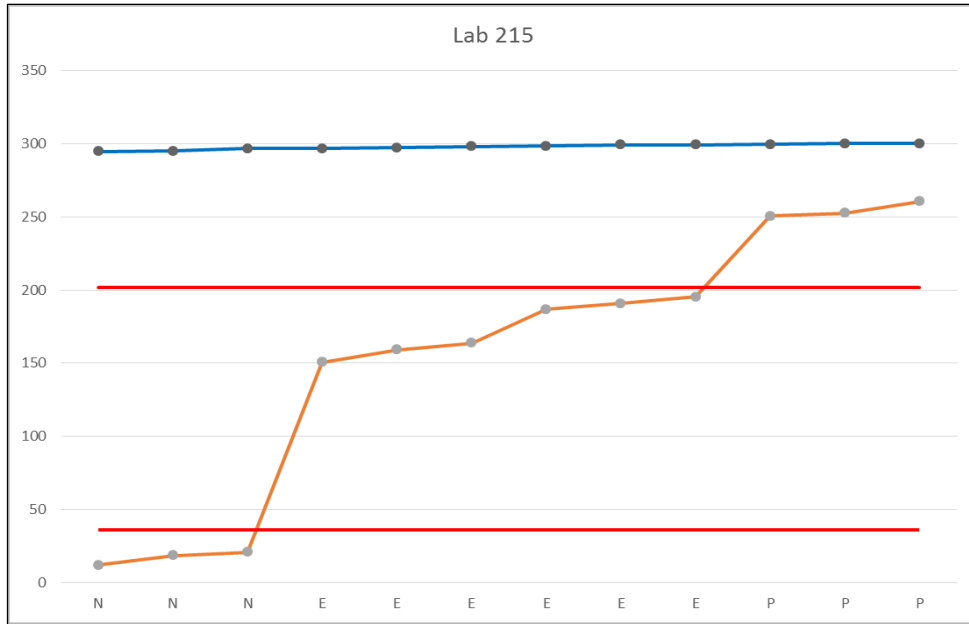
# Individual Laboratory Image Analysis Results

X axis = H-Score. Blue plot = lab results, orange plot = reference lab results



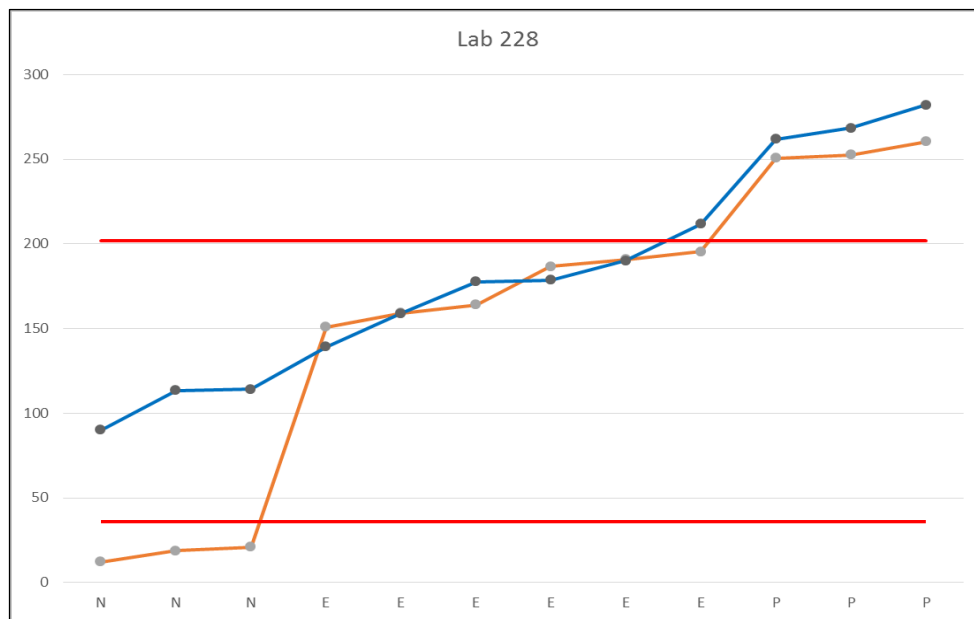
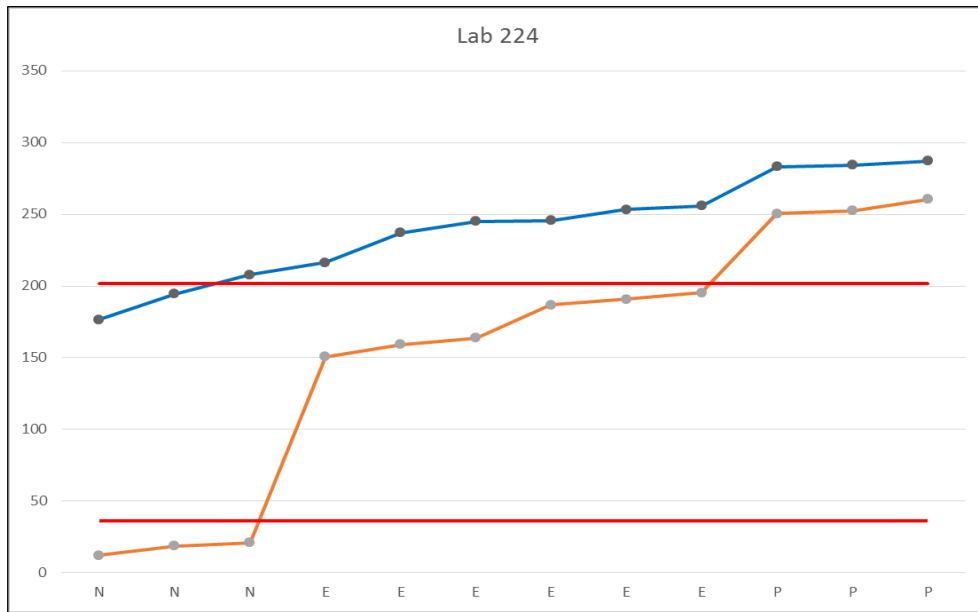
# Individual Laboratory Image Analysis Results

X axis = H-Score. Blue plot = lab results, orange plot = reference lab results



## Individual Laboratory Image Analysis Results

X axis = H-Score.    —●— Blue plot = lab results, —●— orange plot = reference lab results



## Run 60 Protocols (B-Raf v600e)

Labs/Fields	101	114	116	160	175	189	191	193	202	215	217	228
Ag Retrieval Method	CC1	CC1	CC1	CC1	Heat	CC1	CC1	HIER High pH Omnis	citrate pH9.5 Leica	HIER	HIER	HIER
Ag Retrieval Time (min)	64 minutes	32	64 min	64 MIN	64	64	64'	30 min	30 min	64	64	30'
Ab Clone	V600E	VE1	VE1	V600E (VE1)	VE1	VE1	VE1	EP51	V600E	VE1	VE-1	VE1
Ab Dilution	1:200	1/200	RTU	PRE-DILUTED	1 in 2 of predilute	pre-dilute	RTU	RTU	1/100	pre-dilute	RTU	RTU
Ab Supplier/Vendor	Spring Bioscience	Spring Bio	Ventana	VENTANA	Roche	Ventana	Roche	Biospring	Spring	ROCHE	Ventana	Ventana
Ab Lot No.	131216A	131216A	E06579	F00442	F00442	unknown	F00442	n/a	131216e	Fo3208	F05119	F07108
Ab Incubation Time (min)	16 minutes	16	36 min	16 MIN	16	16	16'	36 min	15 min	16	16	15'
Detection System	OptiView	Optiview	Optiview DAB	OPTIVIEW	Optiview polymer Roche	OptiView DAB IHC	optiview	Ventana multimer	Refine Detection kit Leica	OPTIVIEW	Optiview	Leica Bond Refine Detection System
Amplification (Y/N)	Y	Y	N	Y	Y	N	N	Yes	no	Y	Yes	N
Enhancement (Y/N)	Y	Y	.	Y	Y	N	N	Yes	no	N	Yes	N
Chromogen	DAB	DAB	DAB	DAB	DAB	OptiView DAB	DAB	DAB	DAB	DAB	DAB	DAB

Prepared by J.Garratt