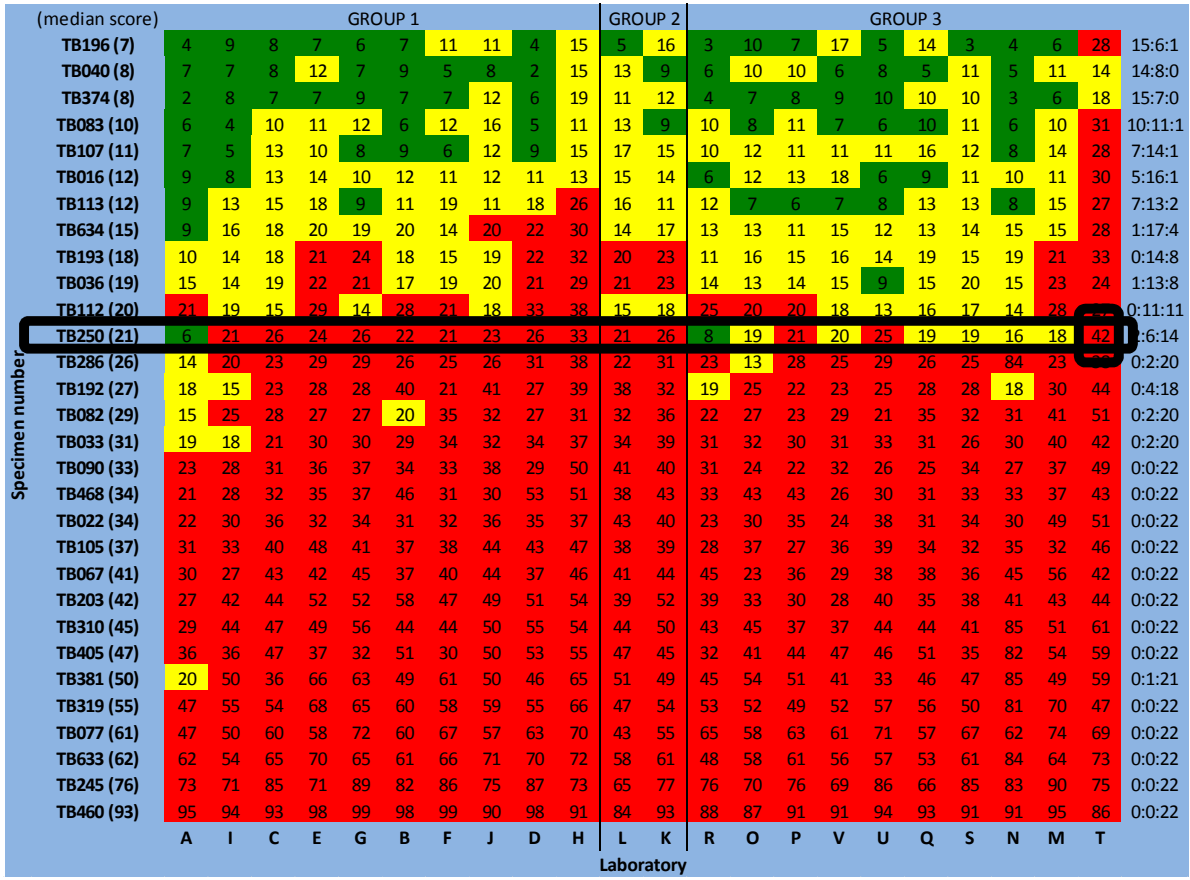
Analytical validation of a standardized scoring protocol for Ki67: phase 3 of an international multicenter collaboration.

Supplemental document: exploratory examination of scoring fields

- **Hypothesis**: detail examination of scoring fields may reveal potential sources of variability that could help explain the observed discrepancies in Ki67 scores
- **Method**: select and examine scoring fields of cases where labs gave fairly discrepant scores
- **Results**: five sources of variability were identified
  1. counting of ductal carcinoma in situ (DCIS)
  2. counting of stromal cells
  3. positive nuclei localized within a part of the scoring field
  4. labs measuring higher or lower than others tended to do so relatively consistently – labs may need recalibration?
  5. hot-spot score discrepancy seems to be driven (partly) by field selection

1. Counting of DCIS

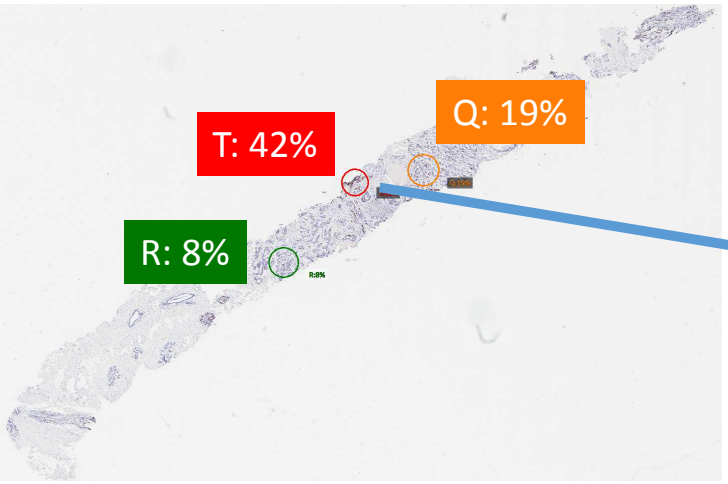
- TB250 (hot-spot scores)
- Lab T gave significantly higher Ki67 score (42%) compared to other labs (*group 3 median: 19%*)
- It appears that DCIS may have been scored. Examination of the corresponding p63 image supported this view (*please refer to the next slide*).

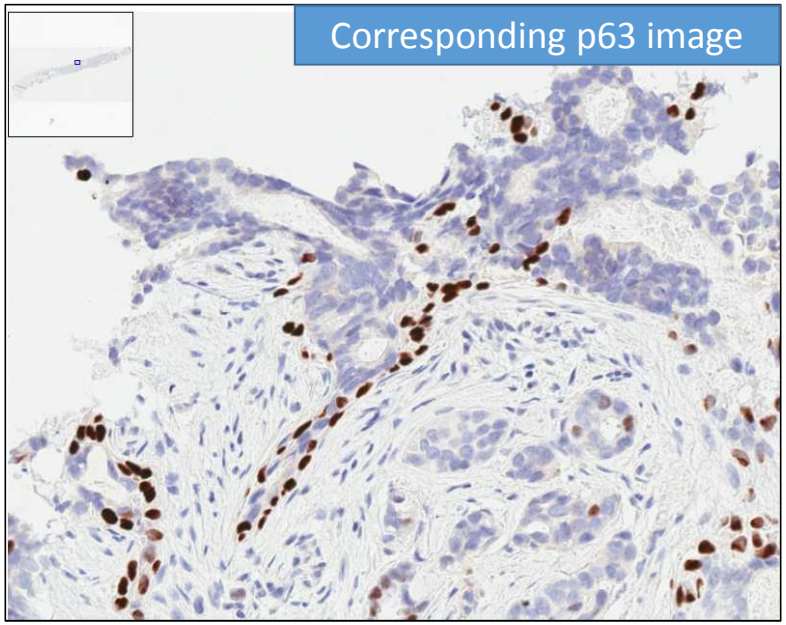


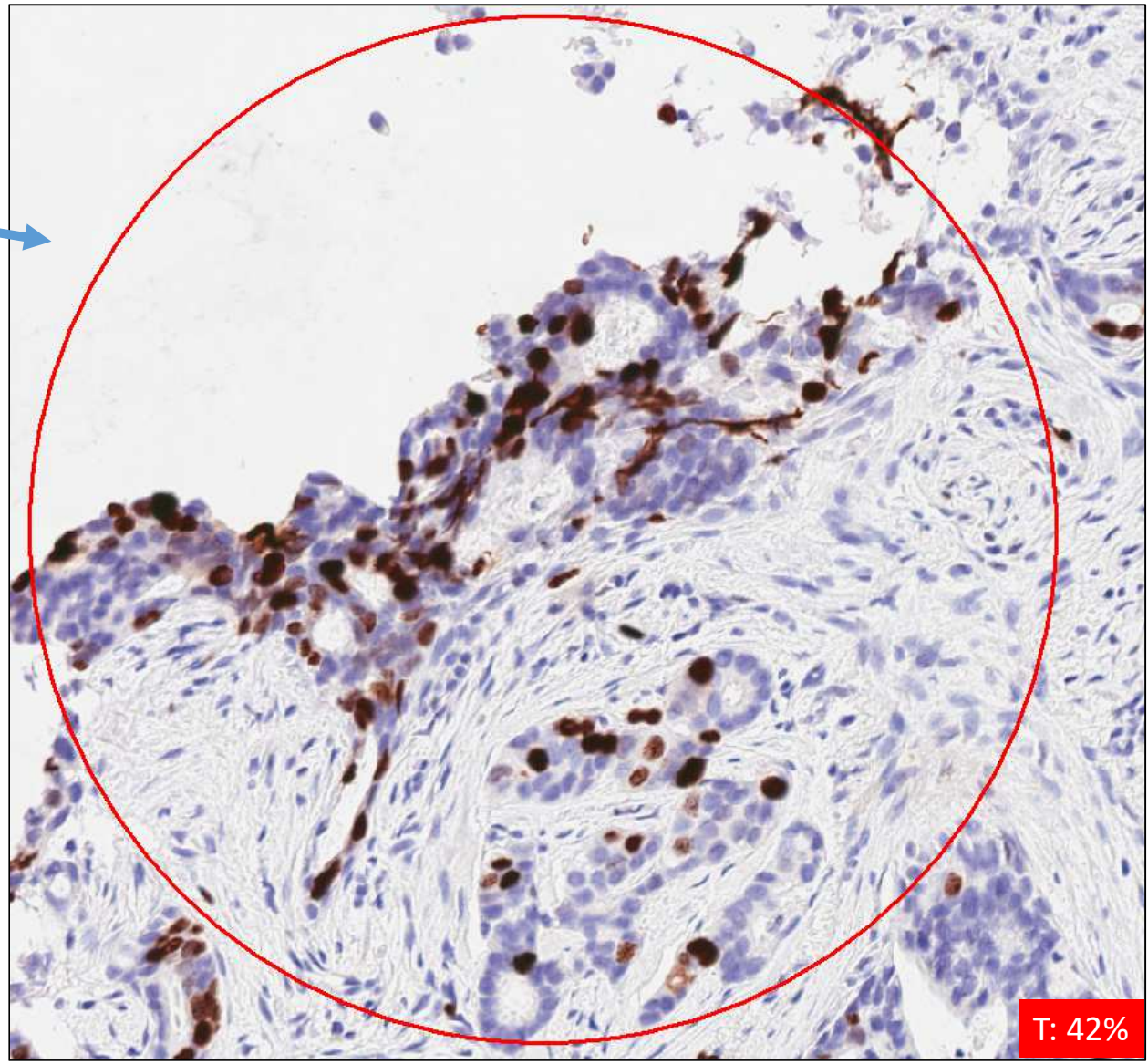
Specimen number (median score)	GROUP 1										GROUP 2		GROUP 3									
	A	I	C	E	G	B	F	J	D	H	L	K	R	O	P	V	U	Q	S	N	M	T
TB196 (7)	4	9	8	7	6	7	11	11	4	15	5	16	3	10	7	17	5	14	3	4	6	28
TB040 (8)	7	7	8	12	7	9	5	8	2	15	13	9	6	10	10	6	8	5	11	5	11	14
TB374 (8)	2	8	7	7	9	7	7	12	6	19	11	12	4	7	8	9	10	10	10	3	6	18
TB083 (10)	6	4	10	11	12	6	12	16	5	11	13	9	10	8	11	7	6	10	11	6	10	31
TB107 (11)	7	5	13	10	8	9	6	12	9	15	17	15	10	12	11	11	11	16	12	8	14	28
TB016 (12)	9	8	13	14	10	12	11	12	11	13	15	14	6	12	13	18	6	9	11	10	11	30
TB113 (12)	9	13	15	18	9	11	19	11	18	26	16	11	12	7	6	7	8	13	13	8	15	27
TB634 (15)	9	16	18	20	19	20	14	20	22	30	14	17	13	13	11	15	12	13	14	15	15	28
TB193 (18)	10	14	18	21	24	18	15	19	22	32	20	23	11	16	15	16	14	19	15	19	21	33
TB036 (19)	15	14	19	22	21	17	19	20	21	29	21	23	14	13	14	15	9	15	20	15	23	24
TB112 (20)	21	19	15	29	14	28	21	18	33	38	15	18	25	20	20	18	13	16	17	14	28	[illegible]
TB250 (21)	6	21	26	24	26	22	21	23	26	33	21	26	8	19	21	20	25	19	19	16	18	42
TB286 (26)	14	20	23	29	29	26	25	26	31	38	22	31	23	13	28	25	29	26	25	84	23	[illegible]
TB192 (27)	18	15	23	28	28	40	21	41	27	39	38	32	19	25	22	23	25	28	28	18	30	44
TB082 (29)	15	25	28	27	27	20	35	32	27	31	32	36	22	27	23	29	21	35	32	31	41	51
TB033 (31)	19	18	21	30	30	29	34	32	34	37	34	39	31	32	30	31	33	31	26	30	40	42
TB090 (33)	23	28	31	36	37	34	33	38	29	50	41	40	31	24	22	32	26	25	34	27	37	49
TB468 (34)	21	28	32	35	37	46	31	30	53	51	38	43	33	43	43	26	30	31	33	33	37	43
TB022 (34)	22	30	36	32	34	31	32	36	35	37	43	40	23	30	35	24	38	31	34	30	49	51
TB105 (37)	31	33	40	48	41	37	38	44	43	47	38	39	28	37	27	36	39	34	32	35	32	46
TB067 (41)	30	27	43	42	45	37	40	44	37	46	41	44	45	23	36	29	38	38	36	45	56	42
TB203 (42)	27	42	44	52	52	58	47	49	51	54	39	52	39	33	30	28	40	35	38	41	43	44
TB310 (45)	29	44	47	49	56	44	44	50	55	54	44	50	43	45	37	37	44	44	41	85	51	61
TB405 (47)	36	36	47	37	32	51	30	50	53	55	47	45	32	41	44	47	46	51	35	82	54	59
TB381 (50)	20	50	36	66	63	49	61	50	46	65	51	49	45	54	51	41	33	46	47	85	49	59
TB319 (55)	47	55	54	68	65	60	58	59	55	66	47	54	53	52	49	52	57	56	50	81	70	47
TB077 (61)	47	50	60	58	72	60	67	57	63	70	43	55	65	58	63	61	71	57	67	62	74	69
TB633 (62)	62	54	65	70	65	61	66	71	70	72	58	61	48	58	61	56	57	53	61	84	64	73
TB245 (76)	73	71	85	71	89	82	86	75	87	73	65	77	76	70	76	69	86	66	85	83	90	75
TB460 (93)	95	94	93	98	99	98	99	90	98	91	84	93	88	87	91	91	94	93	91	91	95	86

TB196 (7)	15:6:1
TB040 (8)	14:8:0
TB374 (8)	15:7:0
TB083 (10)	10:11:1
TB107 (11)	7:14:1
TB016 (12)	5:16:1
TB113 (12)	7:13:2
TB634 (15)	1:17:4
TB193 (18)	0:14:8
TB036 (19)	1:13:8
TB112 (20)	0:11:11
TB250 (21)	:6:14
TB286 (26)	0:2:20
TB192 (27)	0:4:18
TB082 (29)	0:2:20
TB033 (31)	0:2:20
TB090 (33)	0:0:22
TB468 (34)	0:0:22
TB022 (34)	0:0:22
TB105 (37)	0:0:22
TB067 (41)	0:0:22
TB203 (42)	0:0:22
TB310 (45)	0:0:22
TB405 (47)	0:0:22
TB381 (50)	0:1:21
TB319 (55)	0:0:22
TB077 (61)	0:0:22
TB633 (62)	0:0:22
TB245 (76)	0:0:22
TB460 (93)	0:0:22

Laboratory

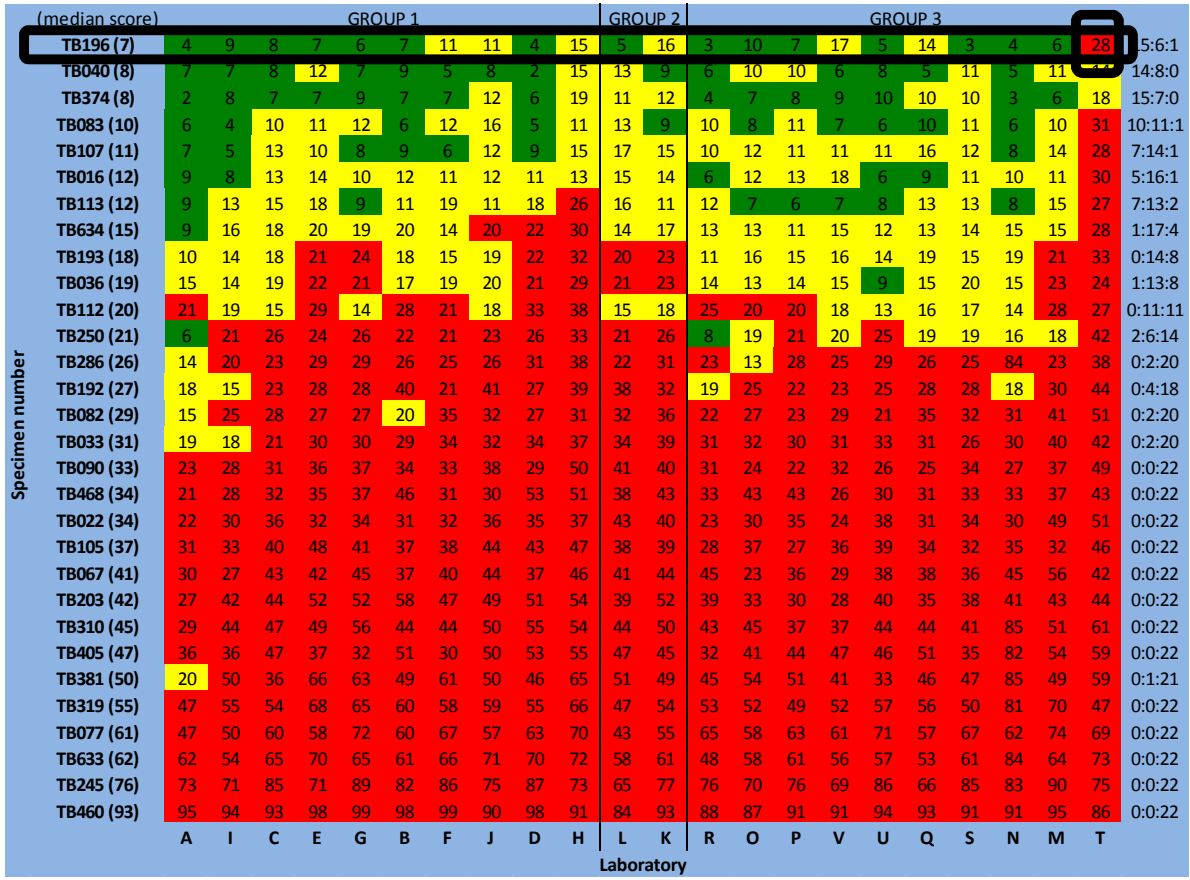

T: 42%
Q: 19%
R: 8%


Corresponding p63 image


T: 42%

2. Counting of stromal cells

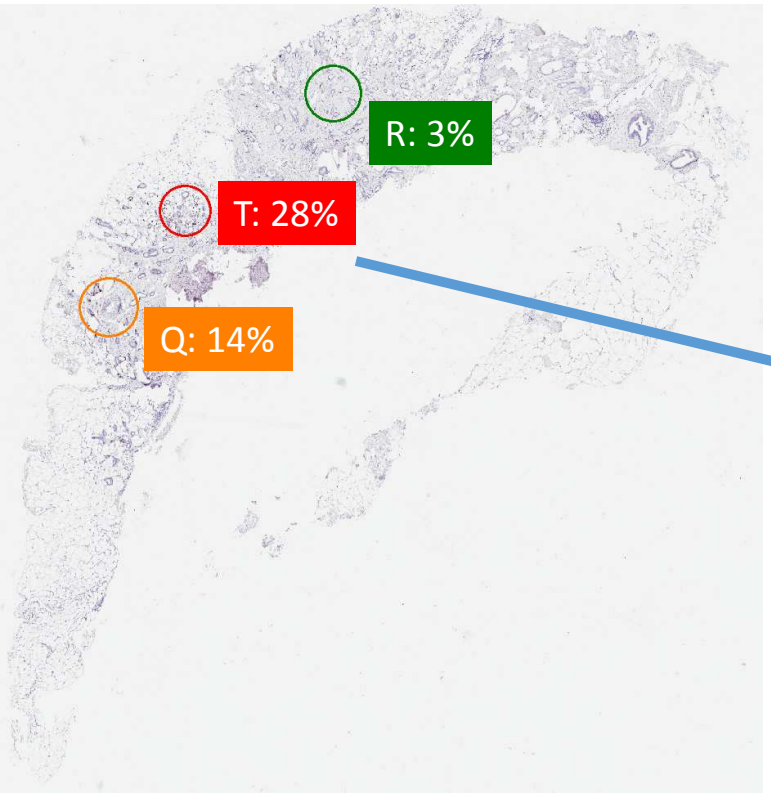
- TB196 (hot-spot scores)
- Lab T gave significantly higher Ki67 score (28%) compared to other labs (*group 3 median: 7%*)
- It seems that some of the stromal cells have been scored, contributing to the higher count (*please refer to the next slide*).

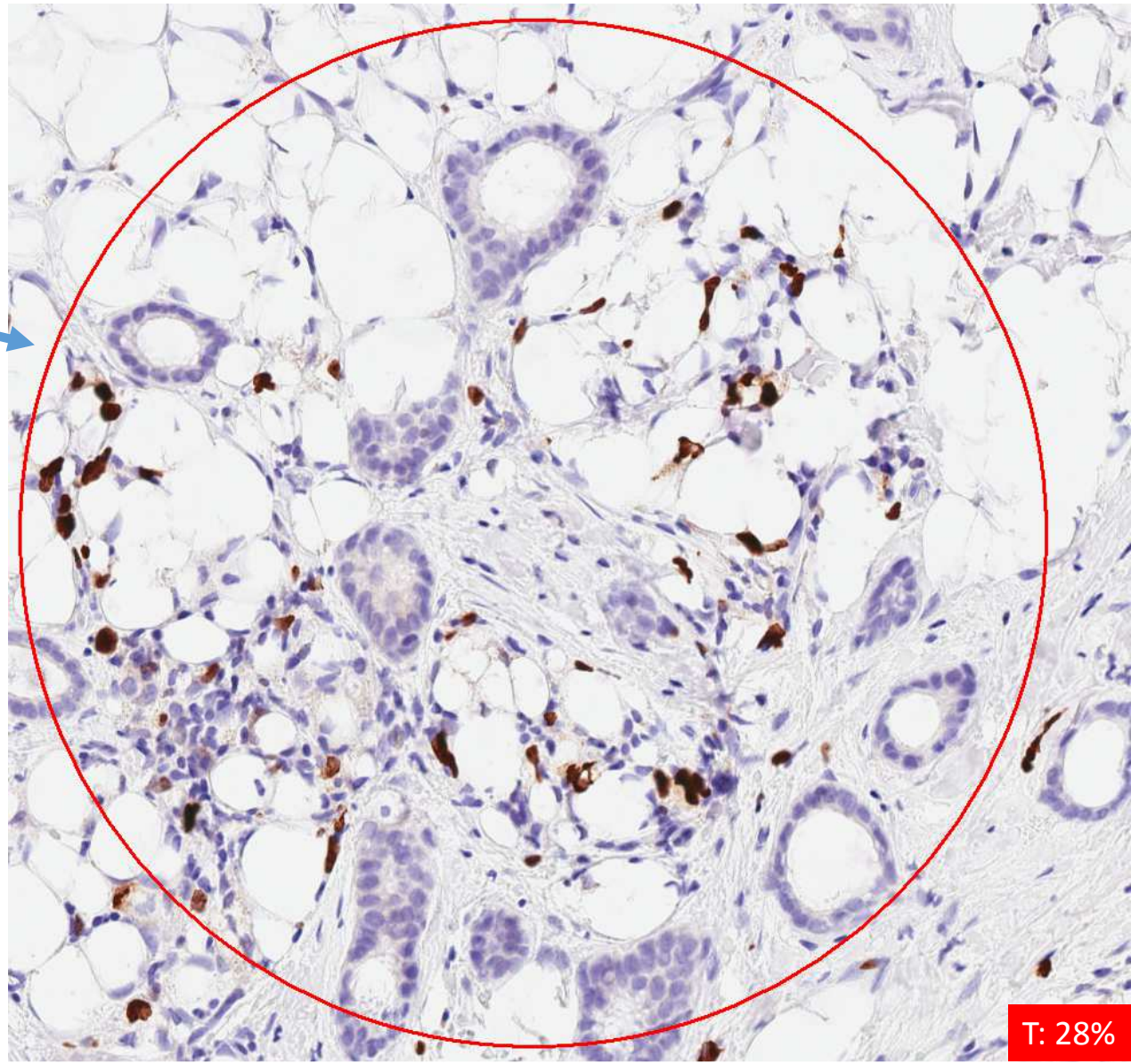


(median score)	GROUP 1										GROUP 2		GROUP 3									
TB196 (7)	4	9	8	7	6	7	11	11	4	15	5	16	3	10	7	17	5	14	3	4	6	28	5:6:1
TB040 (8)	7	7	8	12	7	9	5	8	2	15	13	9	6	10	10	6	8	5	11	5	11	[illegible]	14:8:0
TB374 (8)	2	8	7	7	9	7	7	12	6	19	11	12	4	7	8	9	10	10	10	3	6	18	15:7:0
TB083 (10)	6	4	10	11	12	6	12	16	5	11	13	9	10	8	11	7	6	10	11	6	10	31	10:11:1
TB107 (11)	7	5	13	10	8	9	6	12	9	15	17	15	10	12	11	11	11	16	12	8	14	28	7:14:1
TB016 (12)	9	8	13	14	10	12	11	12	11	13	15	14	6	12	13	18	6	9	11	10	11	30	5:16:1
TB113 (12)	9	13	15	18	9	11	19	11	18	26	16	11	12	7	6	7	8	13	13	8	15	27	7:13:2
TB634 (15)	9	16	18	20	19	20	14	20	22	30	14	17	13	13	11	15	12	13	14	15	15	28	1:17:4
TB193 (18)	10	14	18	21	24	18	15	19	22	32	20	23	11	16	15	16	14	19	15	19	21	33	0:14:8
TB036 (19)	15	14	19	22	21	17	19	20	21	29	21	23	14	13	14	15	9	15	20	15	23	24	1:13:8
TB112 (20)	21	19	15	29	14	28	21	18	33	38	15	18	25	20	20	18	13	16	17	14	28	27	0:11:11
TB250 (21)	6	21	26	24	26	22	21	23	26	33	21	26	8	19	21	20	25	19	19	16	18	42	2:6:14
TB286 (26)	14	20	23	29	29	26	25	26	31	38	22	31	23	13	28	25	29	26	25	84	23	38	0:2:20
TB192 (27)	18	15	23	28	28	40	21	41	27	39	38	32	19	25	22	23	25	28	28	18	30	44	0:4:18
TB082 (29)	15	25	28	27	27	20	35	32	27	31	32	36	22	27	23	29	21	35	32	31	41	51	0:2:20
TB033 (31)	19	18	21	30	30	29	34	32	34	37	34	39	31	32	30	31	33	31	26	30	40	42	0:2:20
TB090 (33)	23	28	31	36	37	34	33	38	29	50	41	40	31	24	22	32	26	25	34	27	37	49	0:0:22
TB468 (34)	21	28	32	35	37	46	31	30	53	51	38	43	33	43	43	26	30	31	33	33	37	43	0:0:22
TB022 (34)	22	30	36	32	34	31	32	36	35	37	43	40	23	30	35	24	38	31	34	30	49	51	0:0:22
TB105 (37)	31	33	40	48	41	37	38	44	43	47	38	39	28	37	27	36	39	34	32	35	32	46	0:0:22
TB067 (41)	30	27	43	42	45	37	40	44	37	46	41	44	45	23	36	29	38	38	36	45	56	42	0:0:22
TB203 (42)	27	42	44	52	52	58	47	49	51	54	39	52	39	33	30	28	40	35	38	41	43	44	0:0:22
TB310 (45)	29	44	47	49	56	44	44	50	55	54	44	50	43	45	37	37	44	44	41	85	51	61	0:0:22
TB405 (47)	36	36	47	37	32	51	30	50	53	55	47	45	32	41	44	47	46	51	35	82	54	59	0:0:22
TB381 (50)	20	50	36	66	63	49	61	50	46	65	51	49	45	54	51	41	33	46	47	85	49	59	0:1:21
TB319 (55)	47	55	54	68	65	60	58	59	55	66	47	54	53	52	49	52	57	56	50	81	70	47	0:0:22
TB077 (61)	47	50	60	58	72	60	67	57	63	70	43	55	65	58	63	61	71	57	67	62	74	69	0:0:22
TB633 (62)	62	54	65	70	65	61	66	71	70	72	58	61	48	58	61	56	57	53	61	84	64	73	0:0:22
TB245 (76)	73	71	85	71	89	82	86	75	87	73	65	77	76	70	76	69	86	66	85	83	90	75	0:0:22
TB460 (93)	95	94	93	98	99	98	99	90	98	91	84	93	88	87	91	91	94	93	91	91	95	86	0:0:22
	A	I	C	E	G	B	F	J	D	H	L	K	R	O	P	V	U	Q	S	N	M	T	

Specimen number

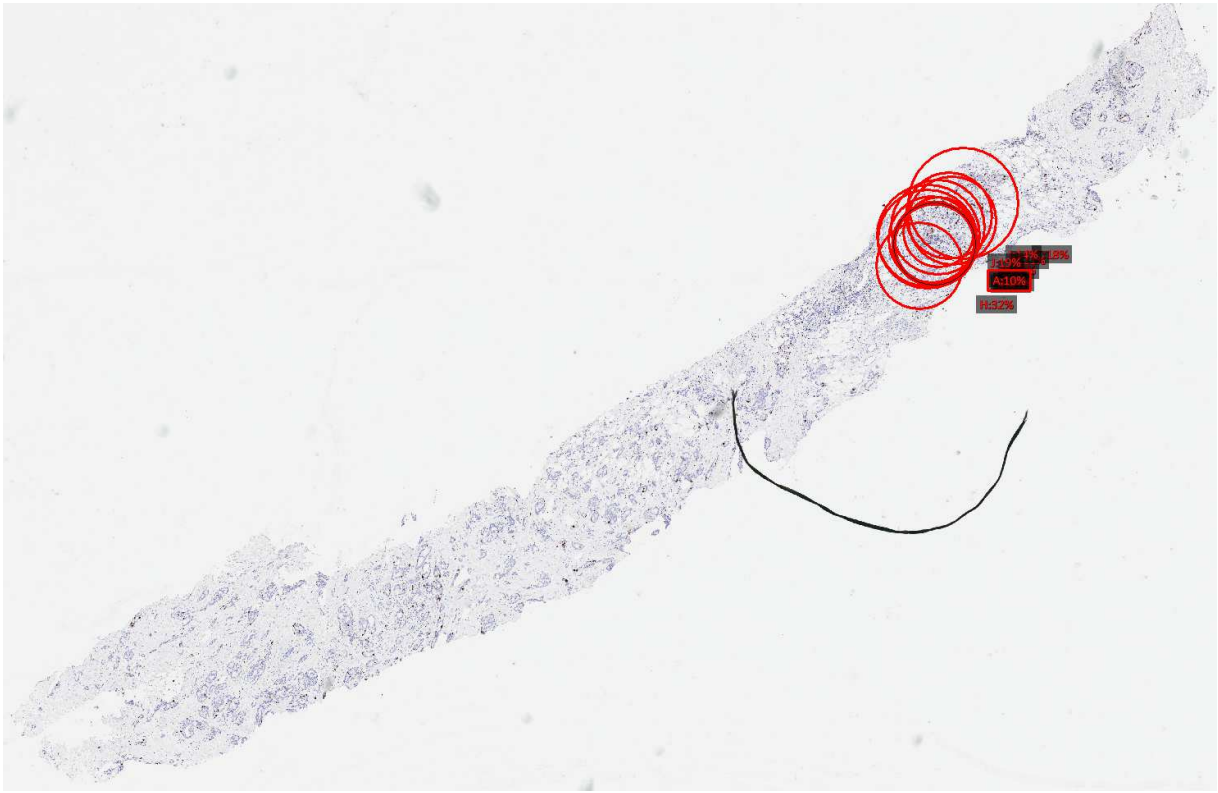
Laboratory

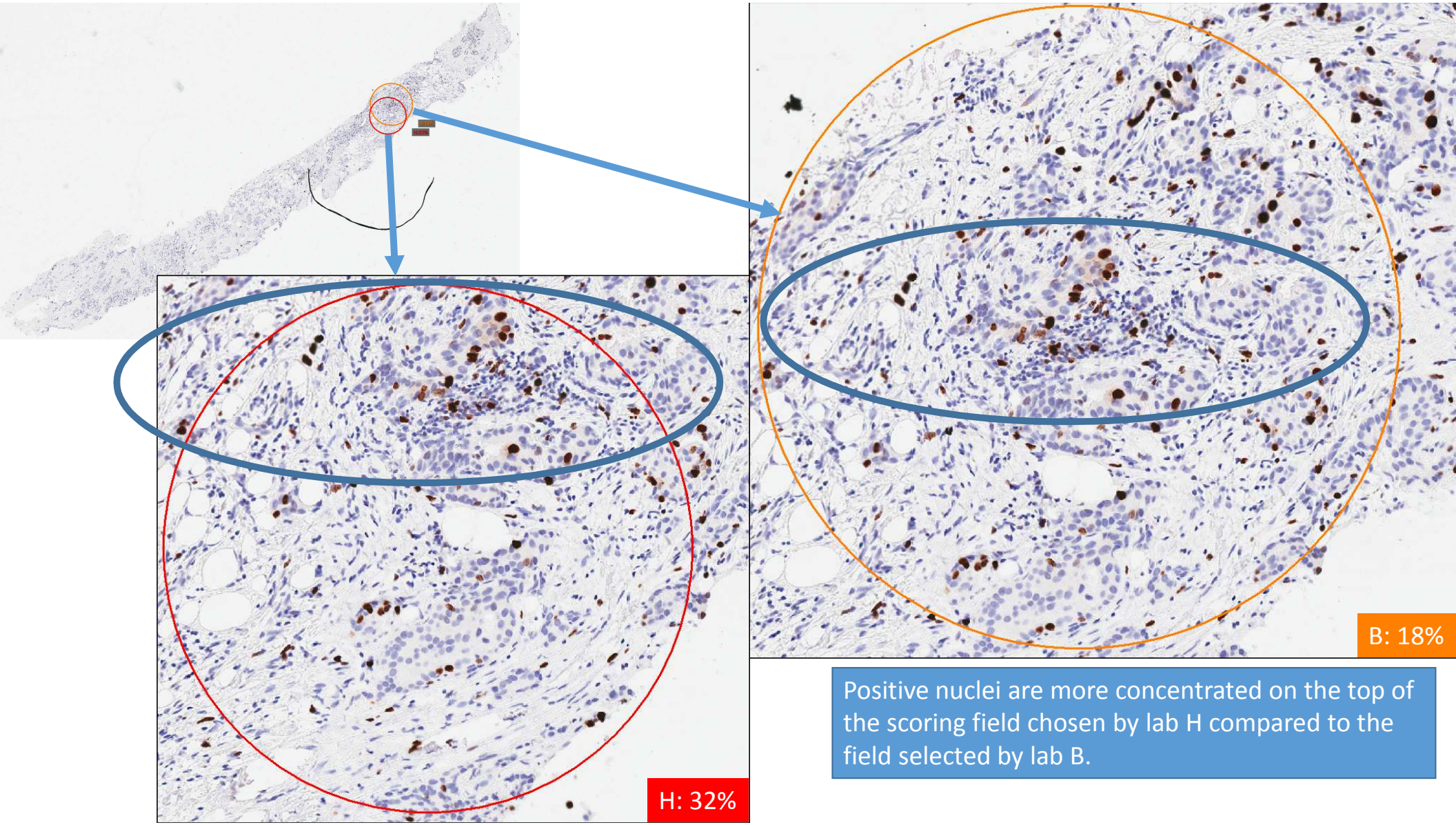

R: 3%
T: 28%
Q: 14%


T: 28%

3. Positive nuclei localized within a part of the scoring field

- TB193 – group 1 slide
- All labs selected a similar hot-spot field.
- However, score among group 1 ranges from 10% to 32% (mean: 19%)
- One possible cause: positive nuclei localized within a part of the scoring field (*please refer to the next slide*).

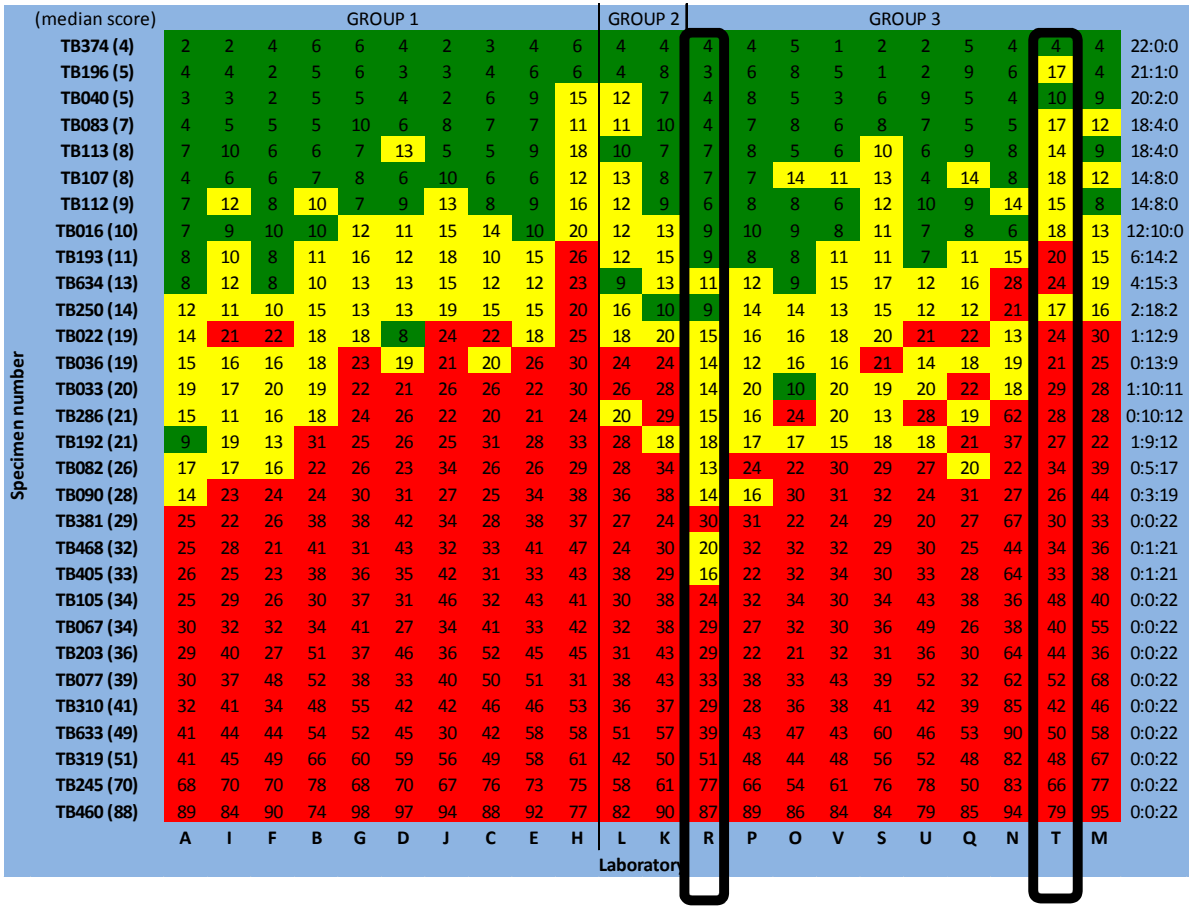




Positive nuclei are more concentrated on the top of the scoring field chosen by lab H compared to the field selected by lab B.

4. Labs measuring higher or lower than others tended to do so relatively consistently – labs may need recalibration?

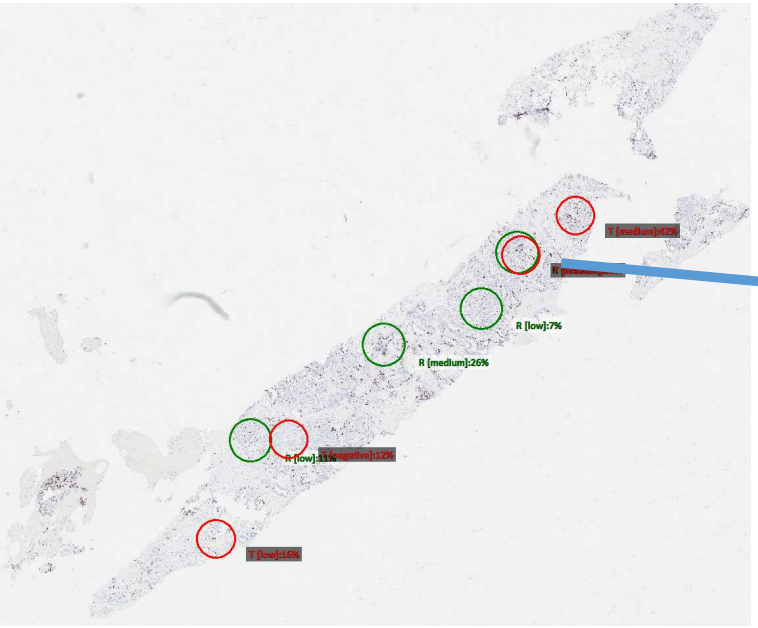
- The following are three cases scored by lab R and T using the global method. They show that the scores given by lab R are fairly consistently lower compared to lab T's scores when they scored the same (or approximately the same) fields.

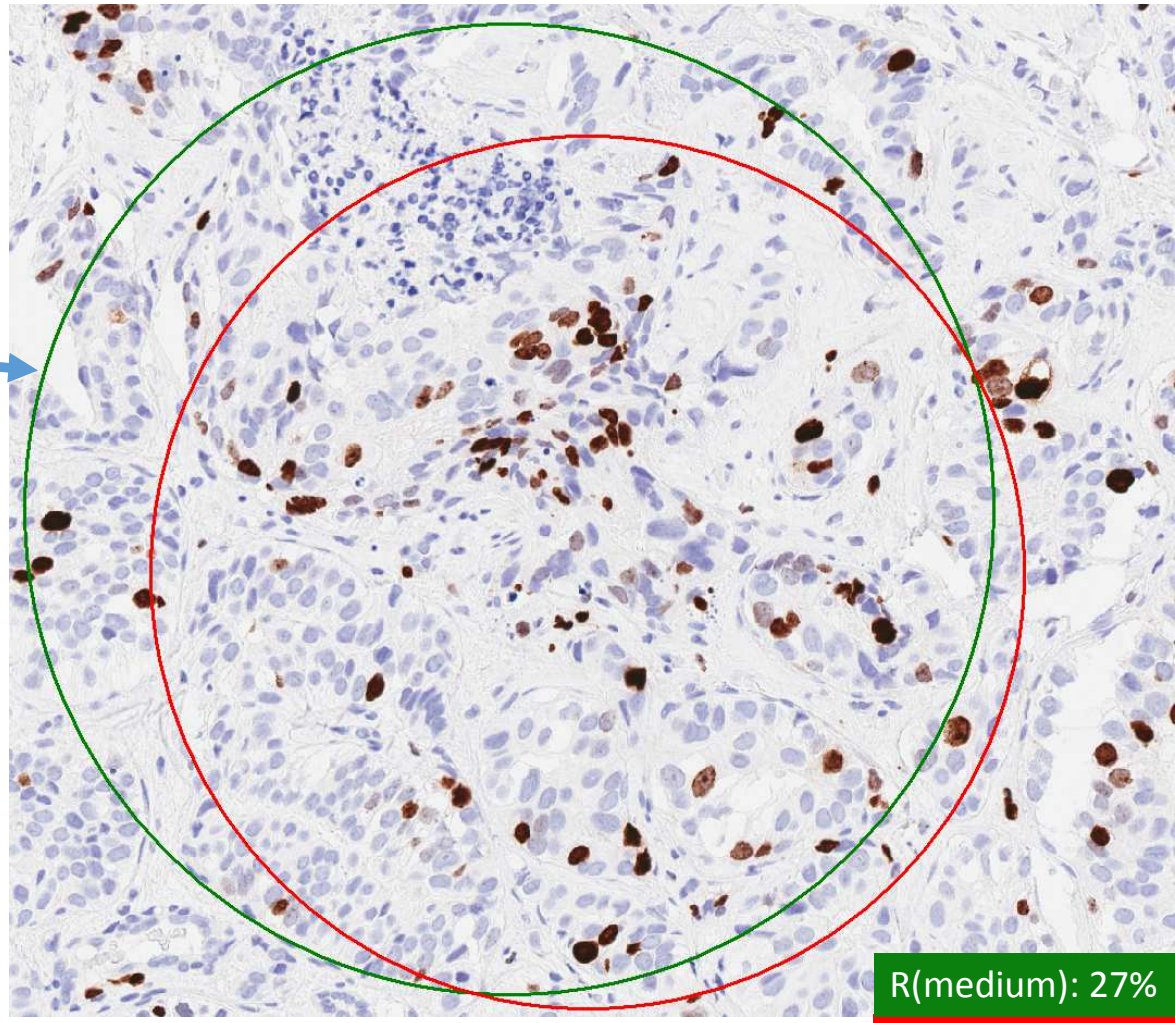


Specimen number	(median score)	GROUP 1										GROUP 2		GROUP 3									
		A	I	F	B	G	D	J	C	E	H	L	K	R	P	O	V	S	U	Q	N	T	M
TB374 (4)	22:0:0	2	2	4	6	6	4	2	3	4	6	4	4	4	4	5	1	2	2	5	4	4	4
TB196 (5)	21:1:0	4	4	2	5	6	3	3	4	6	6	4	8	3	6	8	5	1	2	9	6	17	4
TB040 (5)	20:2:0	3	3	2	5	5	4	2	6	9	15	12	7	4	8	5	3	6	9	5	4	10	9
TB083 (7)	18:4:0	4	5	5	5	10	6	8	7	7	11	11	10	4	7	8	6	8	7	5	5	17	12
TB113 (8)	18:4:0	7	10	6	6	7	13	5	5	9	18	10	7	7	8	5	6	10	6	9	8	14	9
TB107 (8)	14:8:0	4	6	6	7	8	6	10	6	6	12	13	8	7	7	14	11	13	4	14	8	18	12
TB112 (9)	14:8:0	7	12	8	10	7	9	13	8	9	16	12	9	6	8	8	6	12	10	9	14	15	8
TB016 (10)	12:10:0	7	9	10	10	12	11	15	14	10	20	12	13	9	10	9	8	11	7	8	6	18	13
TB193 (11)	6:14:2	8	10	8	11	16	12	18	10	15	26	12	15	9	8	8	11	11	7	11	15	20	15
TB634 (13)	4:15:3	8	12	8	10	13	13	15	12	12	23	9	13	11	12	9	15	17	12	16	28	24	19
TB250 (14)	2:18:2	12	11	10	15	13	13	19	15	15	20	16	10	9	14	14	13	15	12	12	21	17	16
TB022 (19)	1:12:9	14	21	22	18	18	8	24	22	18	25	18	20	15	16	16	18	20	21	22	13	24	30
TB036 (19)	0:13:9	15	16	16	18	23	19	21	20	26	30	24	24	14	12	16	16	21	14	18	19	21	25
TB033 (20)	1:10:11	19	17	20	19	22	21	26	26	22	30	26	28	14	20	10	20	19	20	22	18	29	28
TB286 (21)	0:10:12	15	11	16	18	24	26	22	20	21	24	20	29	15	16	24	20	13	28	19	62	28	28
TB192 (21)	1:9:12	9	19	13	31	25	26	25	31	28	33	28	18	18	17	17	15	18	18	21	37	27	22
TB082 (26)	0:5:17	17	17	16	22	26	23	34	26	26	29	28	34	13	24	22	30	29	27	20	22	34	39
TB090 (28)	0:3:19	14	23	24	24	30	31	27	25	34	38	36	38	14	16	30	31	32	24	31	27	26	44
TB381 (29)	0:0:22	25	22	26	38	38	42	34	28	38	37	27	24	30	31	22	24	29	20	27	67	30	33
TB468 (32)	0:1:21	25	28	21	41	31	43	32	33	41	47	24	30	20	32	32	32	29	30	25	44	34	36
TB405 (33)	0:1:21	26	25	23	38	36	35	42	31	33	43	38	29	16	22	32	34	30	33	28	64	33	38
TB105 (34)	0:0:22	25	29	26	30	37	31	46	32	43	41	30	38	24	32	34	30	34	43	38	36	48	40
TB067 (34)	0:0:22	30	32	32	34	41	27	34	41	33	42	32	38	29	27	32	30	36	49	26	38	40	55
TB203 (36)	0:0:22	29	40	27	51	37	46	36	52	45	45	31	43	29	22	21	32	31	36	30	64	44	36
TB077 (39)	0:0:22	30	37	48	52	38	33	40	50	51	31	38	43	33	38	33	43	39	52	32	62	52	68
TB310 (41)	0:0:22	32	41	34	48	55	42	42	46	46	53	36	37	29	28	36	38	41	42	39	85	42	46
TB633 (49)	0:0:22	41	44	44	54	52	45	30	42	58	58	51	57	39	43	47	43	60	46	53	90	50	58
TB319 (51)	0:0:22	41	45	49	66	60	59	56	49	58	61	42	50	51	48	44	48	56	52	48	82	48	67
TB245 (70)	0:0:22	68	70	70	78	68	70	67	76	73	75	58	61	77	66	54	61	76	78	50	83	66	77
TB460 (88)	0:0:22	89	84	90	74	98	97	94	88	92	77	82	90	87	89	86	84	84	79	85	94	79	95

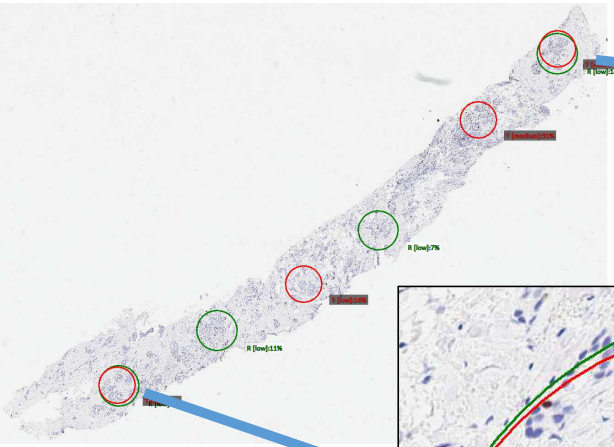
Laboratory

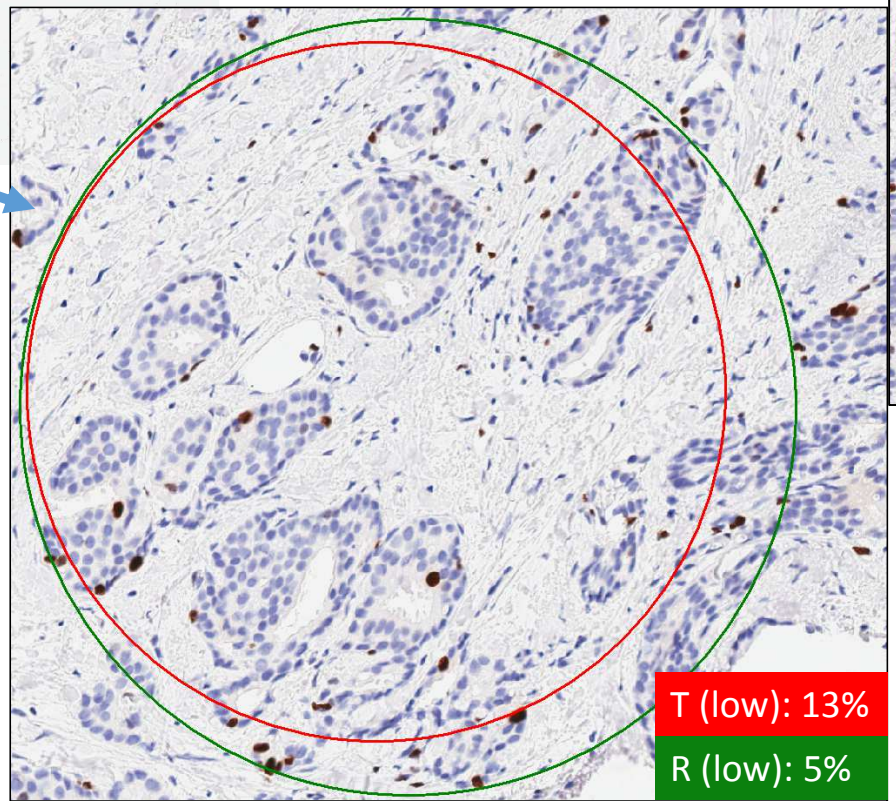
Example case 1 of 3: TB192

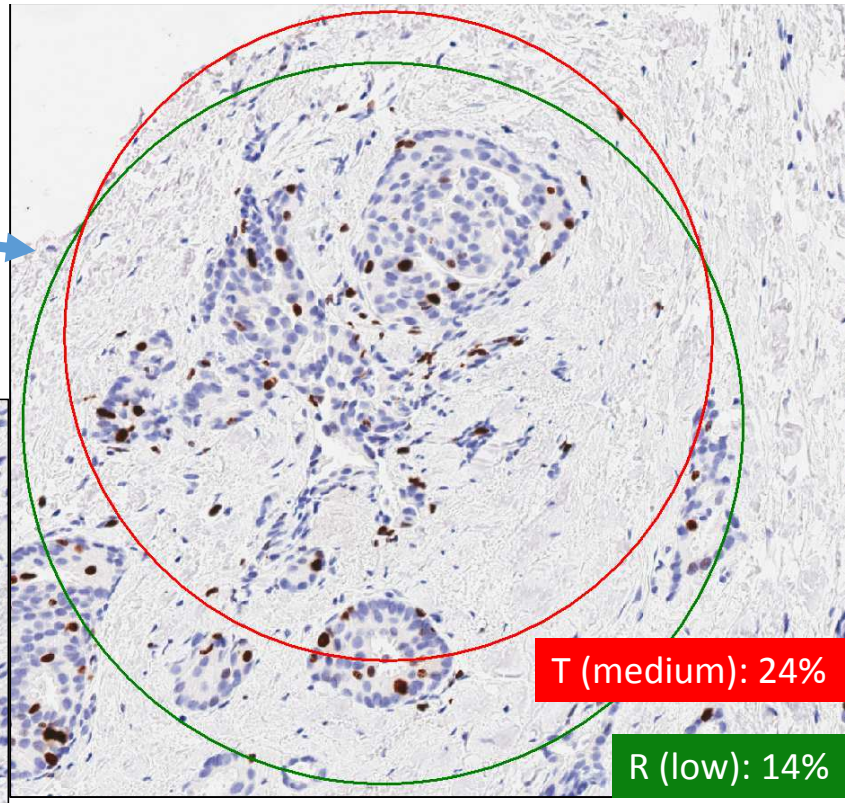




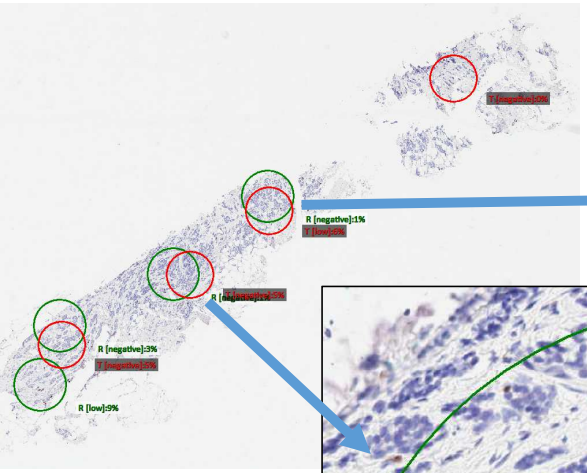
Example case 2 of 3: TB193

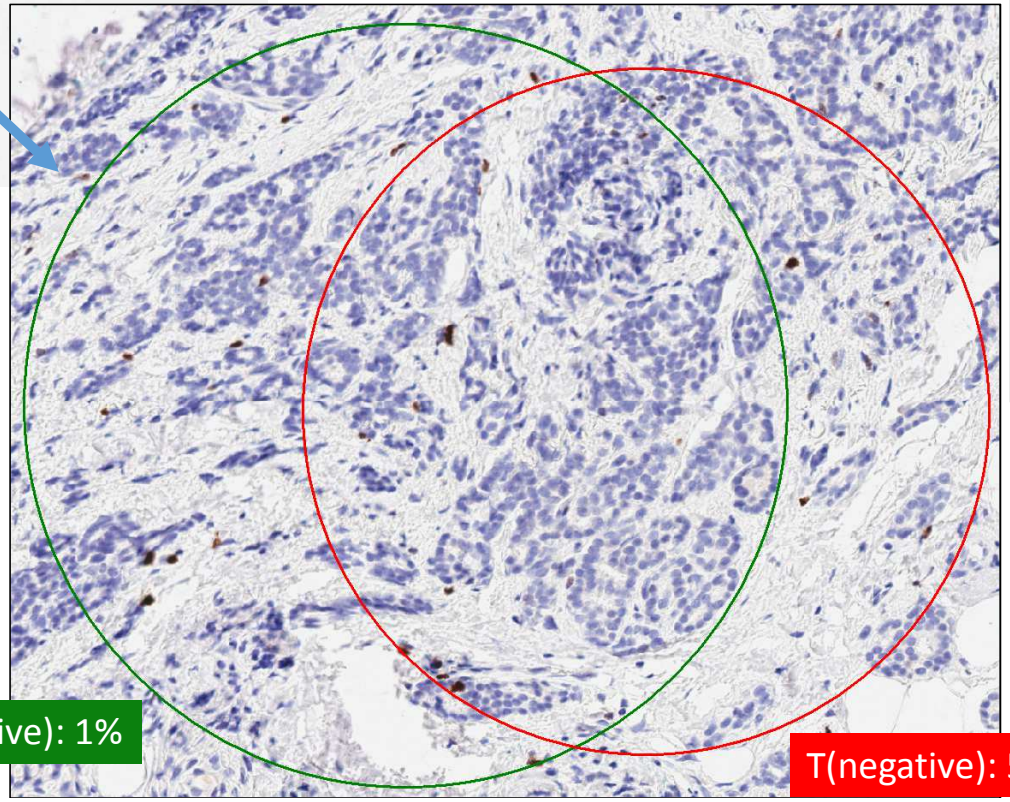


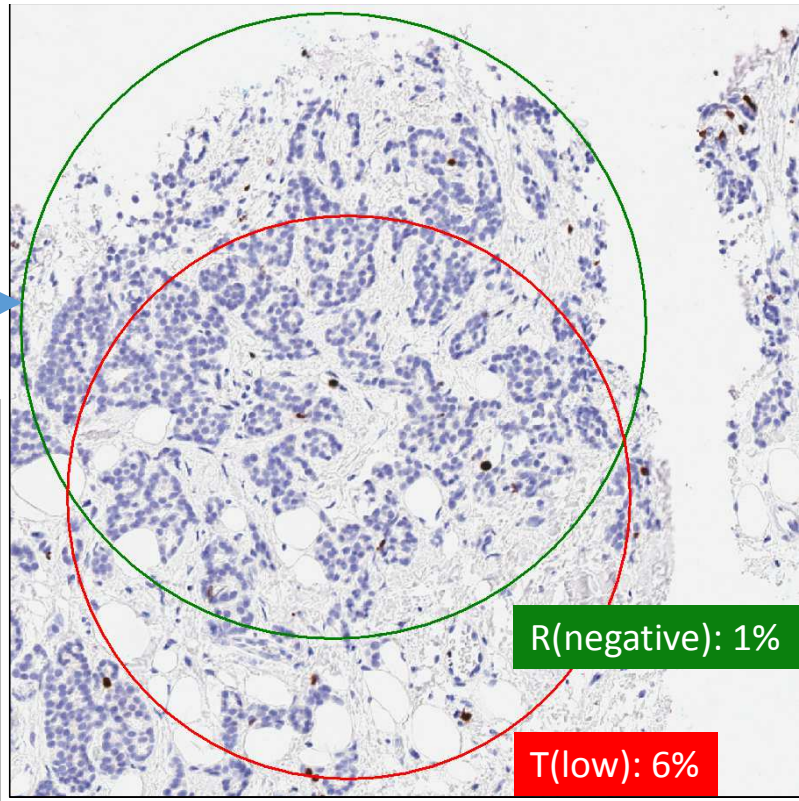




Example case 3 of 3: TB374



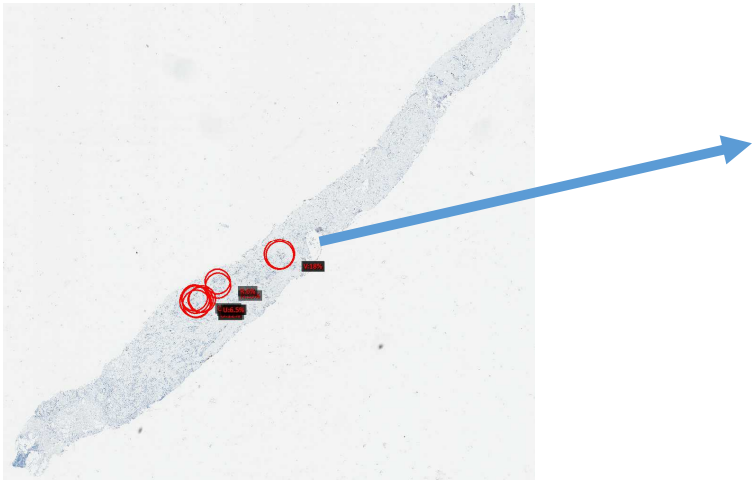


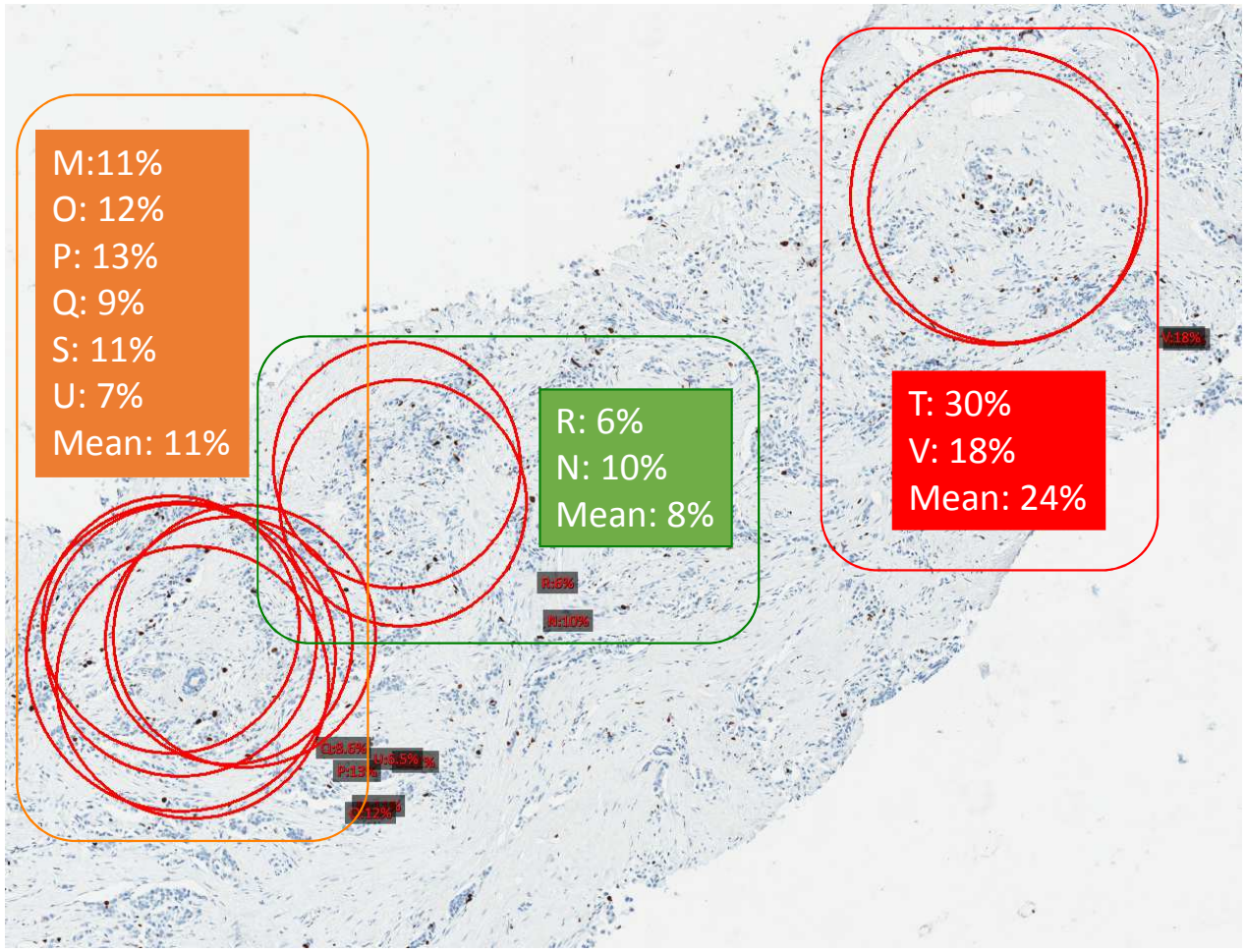


5. Hot-spot score discrepancy seems to be driven (partly) by field selection

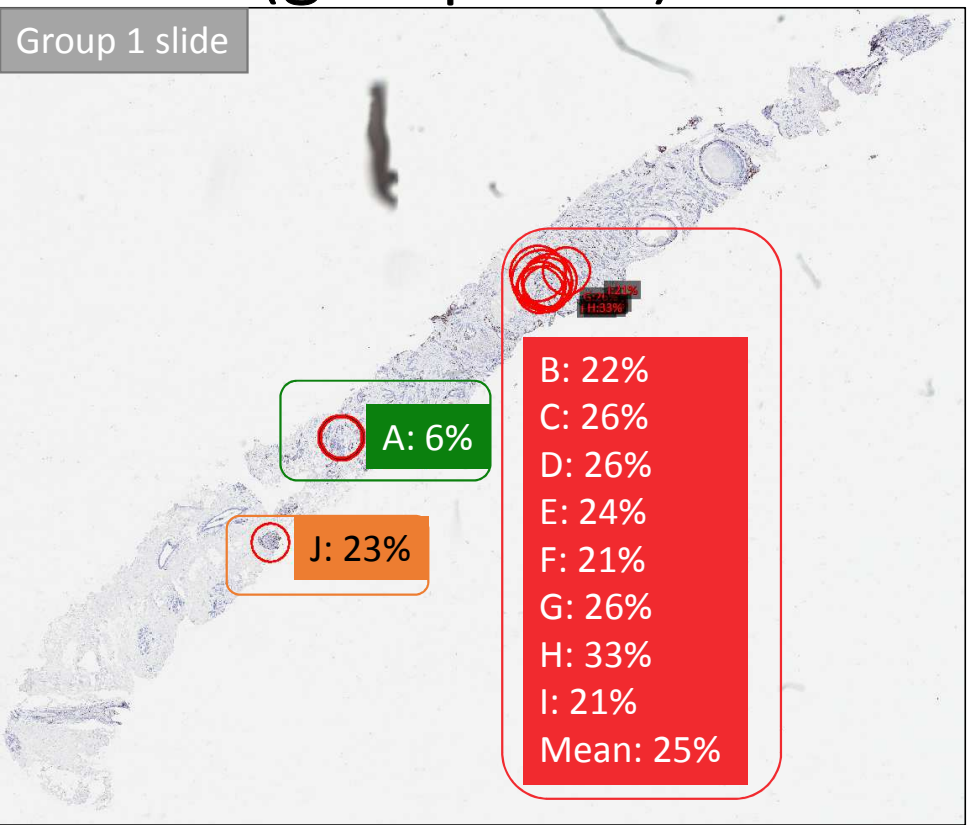
- The following two cases are example of hot-spot scores being fairly consistent if similar hot-spot fields were selected.

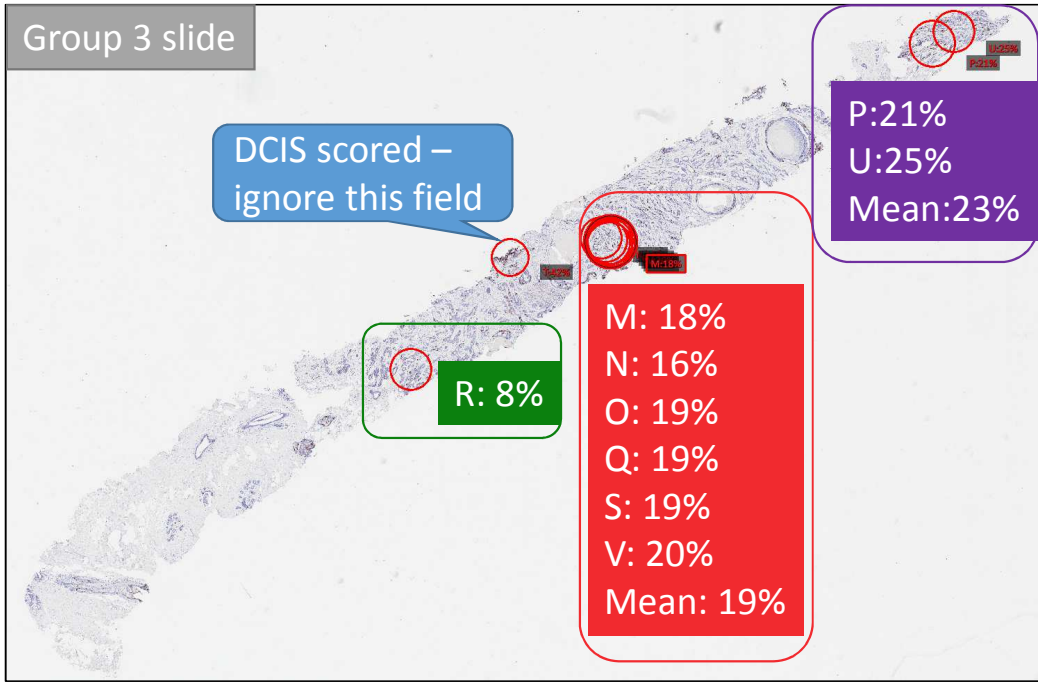
Example 1 of 2: consistent hot-spot scores on similar hot-spot fields.
TB016 (group 3)





Example 2of 2: consistent hot-spot scores on similar hot-spot fields TB250 (group 1&3)





Both lab A (group 1) and R (group 3) scored considerably lower compared to other labs. Apparently, they've selected similar hot-spot fields (albeit in different sections).