

Parameters	Mean ± standard deviation / Percentage
Histological	40.37% (130/322)
Quantity (ng/ul)	43.08 ± 96.93
Quality	5.31 ± 1.23
Average reads for sample	149217.18 ± 106291.64
Mean nr. of mapped reads for sample	147739.80±105672.02
Median read length bp	129.88 ± 4.96
% reads on targets	97.11 ± 3.10
Average reads for amplicon	3382.14 ± 2504.67
% uniformity of coverage	98.01 ± 2.48
Cytological	50.93% (164/322)
Quantity (ng/ul)	48.02 ± 112.03
Quality	5.04 ± 1.63
Average reads for sample	179674.38 ± 137034.82
Mean nr. of mapped reads for sample	178577.14±136158.63
Median read length bp	131.24 ± 6.71
% reads on targets	95.53 ± 12.11
Average reads for amplicon	4158.16 ± 3186.42
% uniformity of coverage	97.61 ± 4.46
Biopsy	73.85% (96/130)
Quantity (ng/ul)	36.98 ± 100.06
Quality	5.33 ± 1.28
Average reads for sample	157269.97 ± 110801.15
Mean nr. of mapped reads for sample	155451.70±110119.56
Median read length bp	130.48 ± 4.41
% reads on targets	97.40 ± 2.44
Average reads for amplicon	3564.42 ± 2607.21
% uniformity of coverage	98.12 ± 2.56
Surgical resection	26.15% (34/130)
Quantity (ng/ul)	61.67 ± 84.04
Quality	5.24 ± 1.02
Average reads for sample	126479.91 ± 88507.90
Mean nr. of mapped reads for sample	125965.03±88400.52
Median read length bp	128.18 ± 5.94
% reads on targets	96.29 ± 4.36
Average reads for amplicon	2867.45 ± 2105.88
% uniformity of coverage	97.70 ± 2.21
FNA	86.59% (142/164)
Quantity (ng/ul)	32.89 ± 68.09
Quality	4.99 ± 1.63
Average reads per sample	174953.35 ± 133103.83
Mean nr. of mapped reads for sample	173905.40±132282.25
Median read length bp	131.04 ± 6.75
% reads on targets	95.88 ± 10.32
Average reads per amplicon	4062.37 ± 3085.26
% uniformity of coverage	97.61 ± 4.24
Brushing + effusion	3.66% (6/164) + 9.76% (16/164) †
Quantity (ng/ul)	143.25 ± 227.71

Quality	6.5 ± 1.26
Average reads for sample	210146.45 ± 156717.21
Mean nr. of mapped reads for sample	208731.08±155581.16
Median read length bp	132.50 ± 6.27
% reads on targets	93.28 ± 19.97
Average reads for amplicon	4772.07 ± 3712.63
% uniformity of coverage	97.67 ± 5.70
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Direct smears	76.22% (125/164)
Quantity (ng/ul)	55.20 ± 125.78
Quality	5.08 ± 1.68
Average reads for sample	190128.67 ± 139967.14
Mean nr. of mapped reads for sample	188937.69±139042.89
Median read length bp	131.38 ± 7.26
% reads on targets	95.25 ± 13.06
Average reads for amplicon	4404.95 ± 3255.70
% uniformity of coverage	97.39 ± 5.05
Cell blocks	23.78% (39/164)
Quantity (ng/ul)	26.48 ± 46.07
Quality	4.87 ± 1.39
Average reads for sample	146167.03 ± 121252.26
Mean nr. of mapped reads for sample	145370.26±120616.56
Median read length bp	130.79 ± 4.47
% reads on targets	96.43 ± 8.29
Average reads for amplicon	3373.49 ± 2815.08
% uniformity of coverage	98.34 ± 1.14
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Diagnosis	
ADC	52.17% (168/322)
Average reads for sample	167496.93 ± 132365.16
Mean nr. of mapped reads for sample	165950.43 ± 131538.59
Median read length bp	129.89 ± 6.99
% reads on targets	95.42 ± 11.92
Average reads for amplicon	3837,92 ± 3092,49
% uniformity of coverage	98,20 ± 2.84
Favor ADC	28.57% (92/322)
Average reads for sample	173212.78 ± 122491,51
Mean nr. of mapped reads for sample	172301.83 ± 121839.69
Median read length bp	132.16 ± 3.79
% reads on targets	97.62 ± 1.75
Average reads for amplicon	4000.08 ± 2847.28
% uniformity of coverage	97.42 ± 4.53
NEC	1.24% (4/322)
Average reads for sample	106203.75 ± 60600,79
Mean nr. of mapped reads for sample	105720.50 ± 60212.82
Median read length bp	130.25 ± 0.83
% reads on targets	96.86 ± 1.07
Average reads for amplicon	2019.23 ± 1824.49
% uniformity of coverage	98.61 ± 0.82
NOS	7.45% (24/322)
Average reads for sample	147817.54 ± 87279.47
Mean nr. of mapped reads for sample	147005.04 ± 86766.32
Median read length bp	130.83 ± 4.75

% reads on targets	96.32 ± 6.48
Average reads for amplicon	3400.36 ± 2057.00
% uniformity of coverage	96.51 ± 5.28
SqCC	1.86% (6/322)
Average reads for sample	136224.00 ± 101344.63
Mean nr. of mapped reads for sample	135063.67 ± 100878.76
Median read length bp	127.50 ± 6.85
% reads on targets	96.86 ± 1.94
Average reads for amplicon	3138.93 ± 2354.12
% uniformity of coverage	96.52 ± 3.61
SITE	
Primary	66.46% (214/322)
Average reads for sample	173895.56 ± 128633.26
Mean nr. of mapped reads for sample	172487.57 ± 127928.48
Median read length bp	130.71 ± 6.07
% reads on targets	96.21 ± 8.72
Average reads for amplicon	3998.57 ± 2988.19
% uniformity of coverage	97.83 ± 3.74
Metastasis	24.84% (80/322)
Average reads for sample	145639.78 ± 113370.71
Mean nr. of mapped reads for sample	144756.08 ± 112583.19
Median read length bp	130.44 ± 5.94
% reads on targets	96.28 ± 10.71
Average reads for amplicon	3313.46 ± 2700.51
% uniformity of coverage	97.68 ± 3.67
Neoplastic cells	
[5%-25%]	28.57% (84 /294)
Average reads for sample	171959.93 ± 116985.92
Mean nr. of mapped reads for sample	172067.95 ± 115310.94
Median read length bp	130.88 ± 7.25
% reads on targets	94.65 ± 15.79
Average reads for amplicon	3992.09 ± 2705.44
% uniformity of coverage	97.84 ± 3.86
[26%-50%]	31.97% (94/294)
Average reads for sample	184656.17 ± 139920.79
Mean nr. of mapped reads for sample	181700.28 ± 114096.12
Median read length bp	131.12 ± 4.50
% reads on targets	97.23 ± 3.38
Average reads for amplicon	4192.93 ± 3284.91
% uniformity of coverage	98.28 ± 2.99
> 50%	39.46% (116/294)
Average reads for sample	147090.67 ± 115542.16
Mean nr. of mapped reads for sample	146200.78 ± 114763.07
Median read length bp	130.07 ± 6.11
% reads on targets	96.56 ± 5.19
Average reads for amplicon	3373.80 ± 2713.05
% uniformity of coverage	97.35 ± 4.09

Supplementary Table 1b. Percentages and characteristic values of the Parameters. We reported only adequate cases

Hypothesis	Hypothesis	p-value (p)
Histological vs. Cytological		
Percentages	40.37% < 50.93%	0.0090 * (CY)
Quantity (ng/ul)	43.08 (ng/ul) < 48.02 (ng/ul)	0.69 (T)
Quality	5.31 > 5.04	0.12 (T)
Average reads for sample	149217.18 < 179674.38	0.0379 * (T)
Mean nr. of mapped reads for sample	147739.80 < 178577.14	0.0345 * (T)
Median read length bp	129.88 < 131.24	0.0009 *(T)
% reads on targets	97.11 > 95.53	0.148 (T)
Average reads for amplicon	3382.14 < 4158.16	0.0236 * (T)
% uniformity of coverage	98.01 > 97.61	0.360 (T)
Biopsy vs. Surgical resection		
Percentages	73.85% > 26.15%	<0.0001* (CY)
Quantity (ng/ul)	36.98 (ng/ul) < 61.67 (ng/ul)	0.20 (T)
Quality	5.33 > 5.24	0.71 (T)
Average reads for sample	157269.97 > 126479.91	0.146 (T)
Mean nr. of mapped reads for sample	155451.70 > 125965.03	0.162 (T)
Median read length bp	130.48 > 128.18	0.0190 * (T)
% reads on targets	97.40 > 96.29	0.071 (T)
Average reads for amplicon	3564.42 > 2867.45	0.163 (T)
% uniformity of coverage	98.12 > 97.70	0.397 (T)
FNA vs. Brushing + effusion		
Percentages	86.59% > 13.42%	<0.0001 * (CY)
Quantity (ng/ul)	32.89 (ng/ul) < 143.25 (ng/ul)	<0.0001 * (T)
Quality	4.99 < 6.5	0.58 (T)
Average reads for sample	174953.35 < 210146.45	0.262 (T)
Mean nr. of mapped reads for sample	173905.40 < 208731.09	0.264 (T)
Median read length bp	131.04 < 132.50	0.342 (T)
% reads on targets	95.88 > 93.28	0.346 (T)
Average reads for amplicon	4062.37 < 4772.07	0.331 (T)
% uniformity of coverage	97.61 < 97.67	0.953 (T)
Direct smears vs. Cell blocks		
Percentages	76.22% > 23.78%	< 0.0001 * (CY)
Quantity (ng/ul)	55.20 (ng/ul) > 26.48 (ng/ul)	0.214 (T)
Quality	5.08 > 4.87	0.480 (T)
Average reads for sample	190128.67 > 146167.03	0.080 (T)
Mean nr. of mapped reads for sample	188937.69 > 145370.26	0.080 (T)
Median read length bp	131.38 > 130.79	0.632 (T)
% reads on targets	95.25 < 96.43	0.596 (T)
Average reads for amplicon	4404.95 > 3373.49	0.077 (T)
% uniformity of coverage	97.39 < 98.34	0.246 (T)
Neoplastic cells		
[5%-25%]; [26%-50%]; > 50%	(28.57%; 31.97%; 39.46%)	0.0165 * (C) 28.57%<31.97%, p = 0.419 (CY) 28.57%<39.46%, p = 0.007 * (CY) 31.97%<39.46%, p = 0.071 (CY)

Average reads for sample	(171959.93; 184686.17; 147090.67)	0.086 (A)
Mean nr. of mapped reads for sample	(172067.95; 181700.28; 146200.78)	0.101 (A)
Median read length bp	(130.88; 131.12; 130.07)	0.418 (A)
% reads on targets	(94.65; 97.23; 96.56)	0.163 (A)
Average reads for amplicon	(3992.09; 4192.93; 3373.80)	0.107 (A)
% uniformity of coverage	(97.84; 98.28; 97.35)	0.198 (A)
Diagnosis		
ADC; Favor ADC; NEC; NOS; SqCC	(52.17%; 28.57%; 1.24%; 7.45%; 1.86%)	<0.0001 * (C) ADC **, p<0.0001 (Z) Favor ADC **, p<0.0001 (Z) NEC***, p<0.0001 (Z) NOS***, p<0.0001 (Z) SqCC***, p<0.0001 (Z)
Average reads for sample	(167496.93; 173212.78; 106203.75; 147817.54; 136224.00)	0.724 (A)
Mean nr. of mapped reads for sample	(165950.43; 172301.83; 105720.50; 147005.04; 135063.67)	0.725 (A)
Median read length bp	(129.89; 132.16; 130.25; 130.83; 127.50)	0.038 * (A) 129.89 < 132.16; p >0.05 (S) 129.89 < 139.25; p >0.05 (S) 129.89 < 130.83; p >0.05 (S) 129.89 > 127.50; p >0.05 (S) 132.16 < 139.25; p >0.05 (S) 132.16 > 130.83; p >0.05 (S) 132.16 > 127.50; p >0.05 (S) 139.25 > 130.83; p >0.05 (S) 139.25 > 127.50; p >0.05 (S) 130.83 > 127.50; p >0.05 (S)
% reads on targets	(95.42; 97.62; 96.86; 96.32; 96.86)	0.499 (A)
Average reads for amplicon	(3937.92; 4000.08; 2019.23; 3400.36; 3138.93)	0.617 (A)
% uniformity of coverage	(98.20; 97.42; 98.61; 96.51; 96.52)	0.154 (A)
SITE: Primary vs. Metastasis		
Percentages	66.46% > 24.84%	< 0.0001 * (CY)
Average reads per sample	173895.56 > 145639.78	0.085 (T)
Mean nr. of mapped reads for sample	172487.57 > 144756.08	0.089 (T)
Median read length bp	130.71 > 130.44	0.733 (T)
% reads on targets	96.21 < 96.28	0.954 (T)
Average reads per amplicon	3998.57 > 3313.46	0.074 (T)
% uniformity of coverage	97.83 > 97.68	0.759 (T)
* = Significant test; T = unpaired t- test; CY = chi-square test with Yates continuity correction; C= multicomparison chi-square test; Z= Z-test; ** = significant most frequent; *** = significant less frequent; A= multicomparison ANOVA test; S=Scheffé's test; T = T-Student test for unpaired data		

Supplementary Table 1b. Statistical tests. All test were performed only on adequate cases