Supplemental Digital Content, containing 2 tables, the Pilot Instructions and Results Form. The Supplemental Digital Content was not copyedited by *Archives of Pathology & Laboratory Medicine.*

Table 1. Summary of SNV Specimens and Performance Results from Reanalyzed SPOT/Dx Pilot Data

																		AACR GENIE DATA		
Variant type	Specimen Number	Gene	Transcript	Nucleotide change	Protein change	No. of labs that tested for variant	No. of labs that detected variant	No. of labs with LOD > mean VAF	No. of corrected false negatives	No. of Miscalls	Percent detected	Engineered VAF, %	Mean Observed VAF, %	SD reported VAF, %	Median Coverage	Range Coverage	WET/DRY Specimen	Variant used in CAP PT	Prevalence in CRC (%)	No. of Cases with Variant	Total No. of Cases in Cohort
SNV	1A	*KRAS*	chr12:25378647T>G	c.351A>C	p.Lys117Asn	21	19	0	2	0	90.5%	5.5	6.1	1.2	2462	137-51574	WET		0.13	20	14328
SNV	1A	*KRAS*	chr12:25380283C>T	c.175G>A	p.Ala59Thr	21	19	0	2	0	90.5%	6.2	5.4	1.0	3900	454-49049	WET		0.19	25	14328
SNV	1B	*KRAS*	chr12:25378647T>G	c.351A>C	p.Lys117Asn	19	16	3	0	0	100.0%	5.0	4.8	0.9	1732	497-46655	DRY		0.13	20	14328
SNV	1B	*KRAS*	chr12:25380283C>T	c.175G>A	p.Ala59Thr	19	17	2	0	0	100.0%	5.0	4.7	0.9	2000	831-29377	DRY		0.19	25	14328
SNV	1C	*KRAS*	chr12:25378647T>G	c.351A>C	p.Lys117Asn	19	19	0	0	0	100.0%	15.0	14.5	1.1	1515	497-46612	DRY		0.13	20	14328
SNV	1C	*KRAS*	chr12:25380283C>T	c.175G>A	p.Ala59Thr	19	19	0	0	0	100.0%	15.0	14.2	1.6	1898	831-29352	DRY		0.19	25	14328
SNV	2A	*KRAS*	chr12:25378647T>A	c.351A>T	p.Lys117Asn	21	18	0	3	0	85.7%	6.3	6.3	0.8	2017	141-44691	WET		0.31	50	14328
SNV	2A	*KRAS*	chr12:25380276T>C	c.182A>G	p.Gln61Arg	21	19	0	2	0	90.5%	7.4	6.9	1.2	3057	252-41051	WET		0.31	41	14328
SNV	2A	*KRAS*	chr12:25398284C>T	c.35G>A	p.Gly12Asp	21	17	0	4	0	81.0%	5.4	5.0	0.7	3751	284-17439	WET	X	12.72	1826	14328
SNV	2B	*KRAS*	chr12:25378647T>A	c.351A>T	p.Lys117Asn	19	18	1	0	0	100.0%	5.0	4.7	0.7	1621	497-46652	DRY		0.31	50	14328
SNV	2B	*KRAS*	chr12:25380276T>C	c.182A>G	p.Gln61Arg	19	17	2	0	0	100.0%	5.0	4.8	0.6	2018	813-29432	DRY		0.31	41	14328
SNV	2B	*KRAS*	chr12:25398284C>T	c.35G>A	p.Gly12Asp	19	18	1	0	0	100.0%	5.0	4.7	0.8	1649	506-14221	DRY	X	12.72	1826	14328
SNV	2C	*KRAS*	chr12:25378562C>G	c.436G>C	p.Ala146Pro	19	19	0	0	0	100.0%	15.0	14.7	1.6	1587	325-34925	DRY		0.08	12	14328
SNV	2C	*KRAS*	chr12:25380276T>C	c.182A>G	p.Gln61Arg	19	19	0	0	0	100.0%	15.0	14.6	1.2	1999	812-29396	DRY		0.31	41	14328
SNV	3A	*KRAS*	chr12:25378562C>G	c.436G>C	p.Ala146Pro	19	17	0	2	0	89.5%	7.5	7.5	2.9	2038	72-33669	WET		0.08	12	14328
SNV	3A	*KRAS*	chr12:25380275T>G	c.183A>C	p.Gln61His	19	16	3	0	0	100.0%	5.3	4.5	0.7	2876	407-57244	WET	X	0.71	103	14328
SNV	3B	*KRAS*	chr12:25380275T>G	c.183A>C	p.Gln61His	19	19	0	0	0	100.0%	15.0	14.5	1.4	1997	812-29317	DRY	X	0.71	103	14328
SNV	3B	*KRAS*	chr12:25398284C>T	c.35G>A	p.Gly12Asp	19	19	0	0	0	100.0%	15.0	14.6	1.4	1517	502-14208	DRY	X	12.72	1826	14328
SNV	3B	*KRAS*	chr12:25378618G>A	c.380C>T	p.Thr127Ile	18	18	0	0	0	100.0%	15.0	14.2	1.4	1524	335-23725	DRY		0.01	1	14328
SNV	3C	*KRAS*	chr12:25378562C>G	c.436G>C	p.Ala146Pro	19	17	2	0	0	100.0%	5.0	4.8	0.5	1713	326-34942	DRY		0.08	12	14328
SNV	3C	*KRAS*	chr12:25380275T>G	c.183A>C	p.Gln61His	19	16	3	0	0	100.0%	5.0	4.8	0.7	2006	812-29339	DRY	X	0.71	103	14328
SNV	4A	*NRAS*	chr1:115252204C>T	c.436G>A	p.Ala146Thr	19	19	0	0	0	100.0%	7.0	7.0	1.0	2376	137-82347	WET	X	0	0	14328
SNV	4A	*NRAS*	chr1:115256528T>G	c.183A>C	p.Gln61His	20	19	0	1	0	95.0%	5.7	6.2	1.0	2021	839-57899	WET	X	0.1	13	14328
SNV	4A	*NRAS*	chr1:115258747C>T	c.35G>A	p.Gly12Asp	20	20	0	0	0	100.0%	6.0	6.1	0.8	2335	765-30399	WET	X	0.86	119	14328
SNV	4B	*NRAS*	chr1:115252204C>T	c.436G>A	p.Ala146Thr	19	19	0	0	0	100.0%	15.0	14.3	1.2	1972	597-43208	DRY	X	0	0	14328
SNV	4B	*NRAS*	chr1:115256536C>T	c.175G>A	p.Ala59Thr	18	18	0	0	0	100.0%	15.0	14.5	1.7	1699	555-28648	DRY		0.02	2	14328
SNV	4B	*NRAS*	chr1:115258747C>T	c.35G>A	p.Gly12Asp	19	19	0	0	0	100.0%	15.0	14.6	2.0	2319	191-34325	DRY	X	0.86	119	14328
SNV	4C	*NRAS*	chr1:115256536C>T	c.175G>A	p.Ala59Thr	18	16	2	0	0	100.0%	5.0	4.8	0.7	2013	401-28663	DRY		0.02	2	14328
SNV	4C	*NRAS*	chr1:115258747C>A	c.35G>T	p.Gly12Val	19	16	3	0	0	100.0%	5.0	4.6	0.7	2647	187-34278	DRY	X	0.11	19	14328
SNV	5A	*NRAS*	chr1:115252289C>A	c.351G>T	p.Lys117Asn	17	13	0	4	1	76.5%	6.2	6.0	0.6	3827	1009-69818	WET		0	0	14328
SNV	5A	*NRAS*	chr1:115256529T>A	c.182A>T	p.Gln61Leu	20	18	0	2	0	90.0%	7.1	6.7	1.2	2858	754-84112	WET	X	0.3	40	14328
SNV	5B	*NRAS*	chr1:115252197G>C	c.443C>G	p.Thr148Ser	18	18	0	0	0	100.0%	15.0	14.6	1.0	1951	600-43193	DRY		0	0	14328
SNV	5B	*NRAS*	chr1:115256529T>A	c.182A>T	p.Gln61Leu	19	16	3	0	0	100.0%	5.0	4.6	0.7	2175	399-28782	DRY	X	0.3	40	14328
SNV	5C	*NRAS*	chr1:115252289C>A	c.351G>T	p.Lys117Asn	15	12	3	0	0	100.0%	5.0	4.8	0.8	2202	1187-29974	DRY		0	0	14328
SNV	5C	*NRAS*	chr1:115256529T>A	c.182A>T	p.Gln61Leu	19	18	0	1	0	94.7%	15.0	14.5	1.9	1985	399-28760	DRY	X	0.3	40	14328
SNV	6A	*NRAS*	chr1:115256536C>T	c.175G>A	p.Ala59Thr	20	19	0	1	0	95.0%	5.6	5.7	1.0	2780	162-58117	WET		0.02	2	14328
SNV	6A	*NRAS*	chr1:115258747C>A	c.35G>T	p.Gly12Val	21	21	0	0	0	100.0%	8.4	7.5	1.0	3179	998-32165	WET	X	0.11	19	14328
SNV	6B	*NRAS*	chr1:115256532C>T	c.179G>A	p.Gly60Glu	19	19	0	0	0	100.0%	15.0	14.6	1.5	2000	401-28513	DRY		0.02	2	14328
SNV	6B	*NRAS*	chr1:115258747C>A	c.35G>T	p.Gly12Val	19	15	4	0	0	100.0%	5.0	3.9	0.5	2779	201-34097	DRY	X	0.11	19	14328
SNV	6C	*NRAS*	chr1:115252204C>T	c.436G>A	p.Ala146Thr	19	16	3	0	0	100.0%	5.0	4.8	0.8	1965	601-43251	DRY	X	0	0	14328
SNV	6C	*NRAS*	chr1:115256528T>G	c.183A>C	p.Gln61His	19	17	2	0	0	100.0%	5.0	4.9	0.6	1998	399-28735	DRY	X	0.1	13	14328
SNV	6C	*NRAS*	chr1:115258747C>T	c.35G>A	p.Gly12Asp	19	16	3	0	0	100.0%	5.0	4.8	0.9	2642	191-34362	DRY	X	0.86	119	14328

Abbreviations: SNV = single nucleotide variant; SPOT/Dx = Sustainable Predictive Oncology Therapeutics and Diagnostics; AACR GENIE = American Association of Cancer Research Genomics Evidence Neoplasia Information Exchange; LOD = limit of detection; VAF = variant allele fraction; SD = standard deviation; CAP = College of American Pathologists; PT = proficiency testing; CRC = Colo-rectal cancer.

Table 2. Summary of MNV Specimens and Performance Results from Reanalyzed SPOT/Dx Pilot Data

																		AACR GENIE DATA		
Variant type	Specimen Number	Gene	Transcript	Nucleotide change	Protein change	No of labs that tested for variant	No of labs that detected variant or	No of labs with LOD > mean VAF	No. of corrected false negatives	No. of Miscalls	Percent detected	Engineered VAF, %	Mean Observed VAF, %	SD reported VAF, %	Median Coverage	Range Coverage	WET/DRY Specimen	Variant used in CAP PT	Prevalence in CRC (%)	No. of Cases with Variant	Total No. of Cases in Cohort
delins/MNV	1A	*KRAS*	chr12:25398281_25398280CG>TT	c.38_39GC>AA	p.Gly13Glu	21	18	1	2	1	90.0%	6.9	6.7	1.1	4019	342-16954	WET	X	0	0	14328
delins/MNV	1B	*KRAS*	chr12:25398281_25398280CG>TT	c.38_39GC>AA	p.Gly13Glu	19	15	4	0	0	100.0%	5.0	4.9	0.5	1808	509-14116	DRY	X	0	0	14328
delins/MNV	1C	*KRAS*	chr12:25398281_25398280CG>TT	c.38_39GC>AA	p.Gly13Glu	19	17	0	2	2	89.5%	15.0	14.2	1.4	1513	505-14055	DRY	X	0	0	14328
delins/MNV	2C	*KRAS*	chr12:25398285_25398283CCA>ACC	c.34_36GGT>TGG	p.Gly12Trp	19	13	0	6	3	68.4%	15.0	14.1	1.1	1779	479-14089	DRY	X	0	0	14328
delins/MNV	3A	*KRAS*	chr12:25398285_25398283CCA>ACC	c.34_36GGT>TGG	p.Gly12Trp	19	13	6	0	0	100.0%	5.1	4.5	0.7	4217	365-17113	WET	X	0	0	14328
delins/MNV	3C	*KRAS*	chr12:25398285_25398283CCA>ACC	c.34_36GGT>TGG	p.Gly12Trp	19	10	9	0	0	100.0%	5.0	4.7	0.5	1887	482-14162	DRY	X	0	0	14328
delins/MNV	4C	*NRAS*	chr1:115258744_115258743CA>TT	c.38_39GT>AA	p.Gly13Glu	15	12	0	3	1	80.0%	5.0	5.1	0.8	2556	187-33441	DRY	X	0	0	14328
delins/MNV	5A	*NRAS*	chr1:115258748_115258746CCA>ACC	c.34_36GGT>TGG	p.Gly12Trp	19	12	2	5	3	70.6%	8.6	7.8	1.1	3724	1012-56912	WET	X	0	0	14328
delins/MNV	5B	*NRAS*	chr1:115258748_115258746CCA>ACC	c.34_36GGT>TGG	p.Gly12Trp	18	8	10	0	0	100.0%	5.0	4.4	0.5	2650	193-33662	DRY	X	0	0	14328
delins/MNV	5C	*NRAS*	chr1:115258748_115258746CCA>ACC	c.34_36GGT>TGG	p.Gly12Trp	18	11	0	7	3	61.1%	15.0	13.8	1.9	2318	191-33471	DRY	X	0	0	14328
delins/MNV	6A	*NRAS*	chr1:115258744_115258743CA>TT	c.38_39GT>AA	p.Gly13Glu	20	15	3	2	1	88.2%	6.7	5.9	1.0	4213	1127-32221	WET	X	0	0	14328
delins/MNV	6B	*NRAS*	chr1:115258744_115258743CA>TT	c.38_39GT>AA	p.Gly13Glu	18	15	0	3	0	83.3%	15.0	14.6	2.1	2326	186-33270	DRY	X	0	0	14328

Abbreviations: MNV = multinucleotide variant; SPOT/Dx = Sustainable Predictive Oncology Therapeutics and Diagnostics; AACR GENIE = American Association of Cancer Research Genomics Evidence Neoplasia Information Exchange; LOD = limit of detection; VAF = variant allele fraction; SD = standard deviation; CAP = College of American Pathologists; PT = proficiency testing; CRC = colorectal cancer; delins = deletions-insertions.