

1 **A Comparison of Different Human Papillomavirus tests in PreservCyt versus SurePath in a Referral**

2 **Population – PREDICTORS 4**

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22 **Running title:**

23 PreservCyt versus SurePath: PREDICTORS 4

24

25 **Key words:** Human papillomavirus testing, cervical screening, PreservCyt, ThinPrep, SurePath.

26

27 **Highlights:**

- 28 • First comparison of HPV tests in PreservCyt and SurePath, 2 samples from each woman
- 29 • Nucleic acid HPV tests showed similar performance in PreservCyt and SurePath
- 30 • Manufacturers' recommended pre-treatment protocols must be observed

31

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38

39 **Abstract**

40 **Background.** Two transport media, PreservCyt and SurePath, are widely used for cervical cytology  
41 screening. There are concerns that they may perform differently for HPV testing.

42 **Objectives.** A comparison of the performance of six different HPV tests in SurePath and PreservCyt  
43 in a referral population using two samples from each woman. The primary goal was to compare the  
44 performance of each test in the two media. Comparisons between assays and viral load  
45 comparisons between media were secondary aims.

46 **Study design.** Two cervical samples were collected in random order at the same visit in women with  
47 abnormal cytology. One sample was placed in 20ml of PreservCyt and the other in 10ml of SurePath.  
48 Aliquots were taken for 4 DNA based tests: *digene* HC2 High-Risk HPV DNA Test, Abbott Realtime, BD  
49 Onclarity and Genera PapType, an RNA based test - Hologic Aptima and a protein test: Oncohealth.

50 **Results.** 630 sample pairs were included in the analyses. For all tests except the protein test  
51 sensitivities were in excess of 90% for CIN2+ and 95% for CIN3+ for both media and with no  
52 significant differences except for a lower sensitivity for CIN2+ of Aptima in SurePath ( 93% vs 98%, P  
53 = 0.005). Specificity for <CIN2 was significantly better in Surepath for HC2, RealTime and Aptima, and  
54 generally lower relative signal strengths were seen with SurePath except for Onclarity, especially  
55 when it was the second sample .

56 **Conclusions.** We found similar sensitivity for CIN3+ in PreservCyt and SurePath for 5 nucleic acid  
57 tests in the two media in a referral population, but signal strength and positivity rates were lower in  
58 SurePath except for the Onclarity test. These results need to be replicated in a screening population.

59

60

61 **Background**

62 Two liquid-based cytology (LBC) systems are commonly used: ThinPrep using PreservCyt transport  
63 medium (Hologic Inc., Marlborough, MA) and SurePath (Becton Dickinson, Sparks, MD) using  
64 SurePath Preservative Fluid. Slide preparation procedures from these media are different<sup>1,2</sup>. Cells are  
65 normally collected using a Cervex-Brush (Rovers Medical Devices, Oss, Netherlands) but in  
66 PreservCyt cells are rinsed into the medium, dispersed by vortexing, and transferred to a microscope  
67 slide after vacuum filtration. In SurePath, the detached head of the brush is placed in the medium.  
68 After initial centrifugation cells are resuspended, put through a density gradient centrifugation with  
69 sampling of the pellet to make the slide. The performance of both systems for cytology is  
70 comparable<sup>1,3</sup>.

71

72 An advantage of LBC is that additional tests, notably HPV, can be run from a single sample, although  
73 only PreservCyt is approved by the FDA for this. Unlike PreservCyt, SurePath contains formaldehyde  
74 to preserve cell morphology and cross-linkage between protein and nucleic acid can occur which can  
75 make DNA undetectable and reduce DNA yield. This is partially reversible using proteinase K (PK)  
76 digestion and/or heat treatment prior to nucleic acid purification<sup>4,5,6</sup>. It is currently unclear whether  
77 such treatment can provide sufficient native HPV DNA/RNA from individual cervical samples for  
78 different HPV assays.

79

80 The majority of early studies of HPV testing in a medium also suitable for cytology have been  
81 conducted using Qiagen's *digene* HC2 High-Risk HPV DNA Test (HC2) in PreservCyt. In a study of 972  
82 SurePath and 1033 PreservCyt screening samples in different women Zhao et al. (2011) found no  
83 significant difference in sensitivity and specificity for the detection of CIN2+ by HC2<sup>7</sup>. A Danish study

84 of 5064 screening samples found the positivity rate correlated moderately well ( $\kappa \geq 0.60$ )  
85 between four assays (HC2(Qiagen), Cobas(Roche), CLART(Genomica) and Aptima(Hologic)) using  
86 SurePath and multiple testing on one sample from each woman<sup>8</sup>. In another study of 367 women  
87 with abnormal cytology similar sensitivities were reported for these four assays<sup>9</sup>. The UK Sentinel  
88 Sites study of 10,051 women referred with borderline or mild dyskaryosis showed a higher overall  
89 HPV positivity rate in PreservCyt than SurePath (68.7% vs 61.7%,  $p < 0.0001$ ). However this may be  
90 confounded by site as all but one site used only one medium and the site using both media found no  
91 significant difference in positive rates<sup>10</sup>. To our knowledge, there has not been a comparison of the  
92 performance of different HPV assays using PreservCyt and SurePath samples collected from the  
93 same woman.

94

## 95 **Objective**

96 The objective of this study was a comparison of the performance of different HPV testing assays in  
97 SurePath and PreservCyt in a routine clinical setting. We used a colposcopy referral population and  
98 compared six HPV assays using two samples from each woman – one collected in PreservCyt and the  
99 other in SurePath. Our primary goal was to compare the performance of each test in the two media.  
100 Comparisons between assays were secondary aims.

101

## 102 **Study design**

103 The study was conducted in the Colposcopy Unit of St. Mary's Hospital, London among women who  
104 had been referred with an abnormal screening result within three months and never treated for CIN.  
105 All women provided written informed consent.

106

107 Two cervical samples were collected with Cervex-Brushes immediately prior to colposcopic  
108 examination in accordance with European guidelines for quality assurance with cervical cancer  
109 screening<sup>11</sup>. To minimise bias, the order of use of transport medium was randomly assigned (1:1).  
110 One brush was agitated in a vial containing 20ml of PreservCyt. The other brush head was removed  
111 and deposited in a vial containing 10ml of SurePath. All samples were stored at 4°C and transferred  
112 within two weeks of collection to the laboratory at the Wolfson Institute of Preventive Medicine,  
113 where HPV testing was performed.

114

115 Within one day of receipt in the laboratory, samples were warmed to room temperature, agitated  
116 for 60 seconds and aliquotted into a fixed order set of tubes, appropriate for six assays. This was  
117 pseudo-randomised to vary the aliquot assigned to each assay by using one of four dispensing  
118 patterns (left to right, right to left, centre to right then centre to left, centre to left then centre to  
119 right). Samples were only identifiable to laboratory staff by participant number. All pathology was  
120 reviewed by M.S. who was blinded to results and participant information.

121

## 122 **Laboratory Methods**

123 Sample storage before testing, aliquot volumes and positivity cut-off values were all in accordance  
124 with the manufacturers' instructions (Table 1). No tests were done on post-gradient pellets.

125 Manufacturers use 'Invalid' or 'Indeterminate' to denote failed results including when a whole plate  
126 or run fails. We refer to all as 'Failed' results in this paper.

127

## 128 **Assays**

129 DNA based:

- 130 • **digene HC2 High-Risk HPV DNA Test:** The QIA Symphony automated platform was used for  
131 nucleic acid extraction with the DSP AXpH DNA Kit (Qiagen, Hilden, Germany). This  
132 consensus DNA test detects a panel of 13 high-risk HPV types  
133 (16,18,31,33,35,39,45,51,52,56,58,59,68). PreservCyt and SurePath samples were processed  
134 using different protocols: PC\_AXpH\_hc2\_V1\_DSP protocol and a modified SP2000\_V1\_DSP  
135 protocol including PK digestion and extended heated lysis time (provided by Qiagen for  
136 research purposes only) respectively<sup>12</sup>. 4ml PreservCyt or 0.5ml of SurePath diluted in 2ml of  
137 deionised water were used. Resulting eluates (60µl) were dispensed into a 96 well  
138 microplate for manual testing. Signal strength was measured in Relative Light Units (RLU)  
139 compared to a reference of approximately 5000 HPV copies.
- 140 • **Abbott RealTime High-Risk HPV assay** used the m2000 processing System (Abbott  
141 Molecular, Abbott Park, Illinois) for the detection of 14 high-risk HPV types, utilising Abbott  
142 reaction vessels as sample input tubes. Types 16 and 18 are individually reported. The  
143 remaining 12 high-risk types are reported together as a  
144 pool(31,33,35,39,45,51,52,56,58,59,66,68).
- 145 • **Becton Dickinson Onclarity HPV Assay** using the BD Viper LT System is a real-time PCR based  
146 DNA test which detects 14 high-risk HPV types. Types 16,18,31,45,51,52 are detected  
147 individually. The remaining eight high-risk types are reported in three groups: (33,58),  
148 (35,39,68) and (56,59,66). A 0.5ml aliquot of thoroughly vortexed SurePath or PreservCyt  
149 was added to 1.7ml of a proprietary HPV diluent. A heat step was employed to ensure that  
150 exfoliated cells were lysed and the sample homogenized prior to extraction of sample  
151 DNA<sup>4,5</sup>.
- 152 • **Genera PapType Test** is a semi-automated, bead-based multiplex full genotyping DNA assay  
153 for 14 high-risk HPV types (16,18,31,33,35,39,45, 51,52,56,58,59,66,68) and two low-risk  
154 HPV types (6,11). The Sirocco platform (Genera Biosystems, Scoresby, Australia) was used.

155 Prior nucleic acid extraction was done using the Abbott m2000sp instrument<sup>13</sup>. Only high-  
156 risk types were considered positive in this study. The assay measure is derived from flow  
157 cytometry and reported as S (signal). Type specific cut-offs were used (Table 1).

158 RNA based:

159 • **Hologic Aptima HPV assay** is based on target capture, transcription-mediated amplification  
160 and hybridization protection for the detection of E6/E7mRNA expression of 14 high-risk HPV  
161 types (16,18,31,33,35,39,45,51,52,56,58,59,66,68). A consensus result for positivity to other  
162 high-risk types was provided. The Direct Tube Sampling platform was used. Typing for 16  
163 and 18/45, available as a reflex test, was not done here. The SurePath sample was treated  
164 with PK at 65°C for 2 hours before being assayed manually. The cut-off was specified to be  
165 0.5 of the ratio of the intensity to the reference standard.

166

167 Protein based assay:

168 • **OncoHealth** (OncoHealth, San Jose, California) protein test is a direct E6/E7 HPV Whole-Cell  
169 ELISA carried out in microtitre wells and is based on detection by non-type specific HPV E6  
170 and E7 monoclonal antibodies<sup>14</sup>. Relative Optical Density (ROD) was used compared to a  
171 reference value of 0.35.

172

173 For all HPV tests except HC2 both samples were processed using an identical assay workflow. Test  
174 details using Preservcyt for HC2, Onclarity, RealTime, PapType and Aptima have been described  
175 previously<sup>13,15,16,17</sup>.

176

177 **Statistical analysis**



178 Primary analyses consisted of paired comparison of the two samples from each woman. For some  
179 assays confounding was observed related to the order in which the sample was taken. Subsequently  
180 additional non-paired analyses by the Wilcoxon Ranksum test and a robust L1 based linear model  
181 with allowance for test order were also conducted<sup>17</sup>. A measure of viral load ( $\log(1+\text{relative intensity}$   
182  $\text{units (RIU)})$  or minus Ct values) was used to perform correlation and regression analyses with  
183 adjustment for sample order for paired samples within each test. Here RIU refers to the signal  
184 strength of the sample compared to a standard (Table 1). Non-amplified samples for Onclarity and  
185 RealTime were given a Ct value of 40 and signal strength 0 for Aptima. SAS (version 9.2) and R  
186 (version 3.2.2) were used. All statistical tests were two-sided and a p-value of 0.05 accepted as  
187 statistically significant.

## 188 **Results**

189 The analysis was based on 630 sample pairs from 652 participating women. Reasons for drop out are  
190 shown in Figure 1. Median age was 30.0 years (IQR=[27.0,34.8]). HC2 was introduced during the  
191 study, and only the last 344 sample pairs were tested. There were no failed results for HC2,  
192 Onclarity, Oncohealth or RealTime. For PapType one sample pair was not tested with either medium  
193 and 46 tests failed (44 sample pairs; 16 in PreservCyt, 30 in SurePath). For Aptima there were 22  
194 failed tests (17 sample pairs; 10 in PreservCyt, 12 in SurePath).

195 Entry cytology was borderline dyskaryosis 193(30.6%), mild dyskaryosis 380(60.4%), moderate  
196 dyskaryosis 37(5.9%) and severe dyskaryosis or glandular abnormality 20(3.2%). A total of  
197 176(28.0%) histology results were CIN2 or worse, including 94(15.0%) cases of CIN3 or CGIN and  
198 2(0.3%) cases of invasive cancer. (Supplementary table S1).

199

200 Overall positivity, sensitivity for CIN3+ and CIN2+ and specificity for <CIN2 for the different tests and  
201 transport media is shown in Table 2. Sensitivity and specificity for CIN2+ is further illustrated in

202 Figure 2 and CIN3+ in Supplementary Figure 1. All tests showed high sensitivities for both samples in  
203 excess of 90% for CIN2+ and 95% for CIN3+, except OncoHealth which had low sensitivity in both  
204 media. A matched-pairs analysis indicated no significant difference between media for sensitivity for  
205 either CIN2+ or CIN3+ for any test, except for Aptima which was slightly less sensitive in SurePath  
206 (98% vs 93%,  $P=0.005$ ). However, there were differences in specificity with significantly higher  
207 specificities for HC2, RealTime, Aptima and OncoHealth in SurePath. Although showing some  
208 predictive ability above chance in PreservCyt, the OncoHealth test was substantially and significantly  
209 less sensitive than all other tests ( $\leq 60\%$  for both media for both CIN2+ and CIN3+), but was more  
210 specific than the other assays. There was no significant difference however with the OncoHealth  
211 assay between media.

212 Signal strength (viral load estimate) differed by transport medium and test order (Table 3). Little  
213 difference was seen between the two media when used as a first test, except for substantially higher  
214 values for RealTime in SurePath ( $P < 2 \times 10^{-5}$ ) and HC2 in PreservCyt ( $P=0.009$ ). For HC2 this probably  
215 reflects a larger sample volume for PreservCyt. Significantly higher values were seen for PreservCyt  
216 (vs SurePath) when both were used as a second sample especially for HC2, again with the exception  
217 of Onclarity. Type specific results for HPV16 and 18 for RealTime, Onclarity and PapType gave a  
218 similar pattern (Table 3).

219 PreservCyt values were not statistically significantly different between the first versus second  
220 samples in all cases except for OncoHealth where they were substantially lower in the second  
221 sample (Table 3). For SurePath, significant differences between the first and second sample were  
222 seen for all tests, but the second sample gave higher levels for RealTime and Onclarity and lower  
223 levels for the other tests. For HC2 the RLU values were much lower in the second sample for  
224 SurePath, possibly due to the smaller sample volume.

225 Correlation between signal strength measurements for the two media for each test is shown in Table  
226 4. While correlations for the tests in the two media were quite good, except for OncoHealth, the

227 slopes were significantly less than unity for all tests except HC2 which was 0.966 ( $p=0.23$ ), indicating  
228 that the values are generally higher for PreservCyt. Minimal correlation between media could be  
229 seen for the OncoHealth test. A fuller presentation of the differences between the two media is  
230 shown as scatterplots for each test in Supplementary Figures S2-7, in which the order of the test is  
231 also depicted.

232

### 233 **Discussion**

234 Our results indicate that similar sensitivities and specificities can be achieved with either PreservCyt  
235 or SurePath for 5 of the 6 HPV tests, provided that the manufacturer's recommended pre-  
236 treatments are observed. Some loss of sensitivity for CIN2+ was seen for RealTime and Aptima in  
237 SurePath, but this was minimal for CIN3+. The largest differences were seen for specificity which was  
238 generally better for SurePath, especially for HC2, RealTime and Aptima. This is likely to also be true  
239 for primary screening but direct verification in this setting is needed. Poor performance was seen for  
240 the Oncohealth protein test in both media. This protein-based test however is known to be less  
241 stable in alcohol and a second generation test has been developed since this study was carried out.

242 The failure rate for PapType was relatively high (3.6%, 45/1260). No specific reason could be  
243 identified, but this was a prototype test with the complexity of full typing, so improvements are  
244 likely in the future. The failure rate was 1.3% for Aptima, but there were no failures for other tests.

245 The differences between tests were greater for the second than the first sample, illustrating the  
246 differences in a true diagnostic situation where only a first sample would be used. This highlights the  
247 need for an adequate sample and may be a factor in the discordant results between assays as found  
248 by Rebolj et al (2014)<sup>8</sup>. The SurePath vial contained 10mls and PreservCyt 20mls of transport  
249 medium, thus concentration of cells in SurePath is greater than PreservCyt. The only test where the  
250 amount of DNA in the tested sample would be expected to be the same in both media would be the

251 Aptima test where the aliquot volume was 1ml of PreservCyt and 0.5ml of SurePath. All others tests  
252 except HC2 used an equal aliquot volume (0.5ml) and would lead to less DNA in the PreservCyt  
253 sample. For HC2 4mls were assayed from PreservCyt versus 0.5ml from SurePath. However this had  
254 no measurable impact on the results.

255 Although not of direct clinical relevance, comparison of the quantitative measures of signal strength  
256 as a surrogate measure of viral load provides additional insight into the comparative performance of  
257 the different tests in the two transport media. We recognise several confounding factors to this  
258 measure including cell number and specific methods of measuring signal strength. In general lower  
259 signal strength values were obtained for SurePath. The largest differences were seen for HC2  
260 potentially partly attributable to smaller sample volume for SurePath.

261 Most HPV assays have been more fully optimized for PreservCyt, which has been in use for longer.  
262 An exception is the Onclarity assay, developed by the manufacturer of SurePath. The Onclarity assay  
263 uses a heat step in sample pre-processing for both sample types and little difference between media  
264 was seen. At the time of this study no HPV test manufacturer had an approved protocol for their  
265 assay in the SurePath medium and it is possible that this will impact on performance.

266 In summary this prospective study is the first comprehensive comparison of a range of HPV tests in  
267 the two most commonly used LBC transport media, where two samples are taken from each woman.  
268 No major differences in performance were seen when the manufacturer's protocols were used.  
269 These tests have all performed well in this referral population and although all appear suitable for  
270 screening they need to be validated in a screening population using Arbyn's criteria<sup>11</sup>.

271

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274 who took the time to read our information and in particular those who consented to take part.

275

276 **Conflicts of Interests**

277 Funding: funded from Cancer Research UK Programme grant C569/A10404, and supplemented by  
278 financial contributions and assay kits from Qiagen, BD, Abbott, Genera, Hologic and Oncohealth.

279 Competing interests: JC has received honoraria for lectures from Abbott and Qiagen and served on  
280 advisory boards for Hologic and BD.

281 All authors have attended meetings with manufacturers of HPV assays but none was compensated  
282 for their work on this project.

283 All manufacturers had the right to comment on a draft version of this manuscript, but had no  
284 involvement in the final content or decision to publish.

285 Ethical approval: Received in August 2011 from NHS Health Research Ethics Service Committee  
286 London–Hampstead [Reference 11/LO/1147].

287

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343

344 **Table 1. HPV assays performed, positivity cut-off and aliquot volume.**

Test	Positivity Cut-off <sup>a b</sup>	Aliquot volume (ml)	
		PreservCyt	SurePath
HC2	≥ 1 RLU	4.0	0.5
RealTime	≤ 32 Ct	0.5	0.5
Onclarity	≤ 34.2 Ct	0.5	0.5
PapType	HPV58 ≥ 0.0004		
	HPV68 ≥ 0.0003	0.5	0.5
	All others ≥ 0.0002		
Aptima	≥ 0.5 RIU	1.0	0.5
OncoHealth	≥ 0.35 OD	1.0	1.0

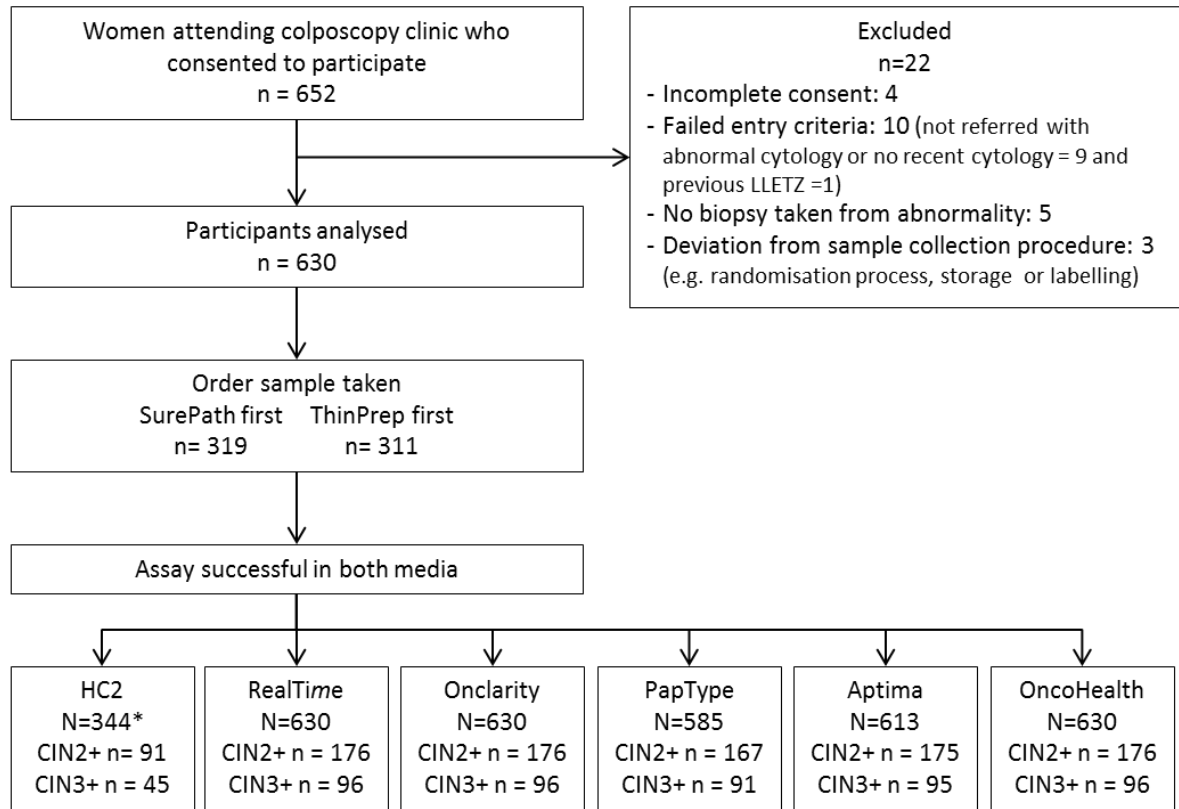
345 <sup>a</sup> For all tests except RealTime and Onclarity, units are ratio of signal strength to reference standard

346 <sup>b</sup> RLU – relative light units; Ct – cycle threshold; RIU – relative intensity units; OD –optical density

347



Figure 1: CONSORT diagram of patient enrolment and number with HPV testing by different tests



349

\*fewer due to late entry into study – no failed tests

350 **Table 2. Overall positivity, sensitivity for CIN3+ and CIN2+, specificity for < CIN2 and agreement for**  
 351 **different tests and transport media.**

	<b>Overall positivity (%)</b>	<b>Sensitivity</b>		<b>Specificity &lt;CIN2 (N = 454)<sup>c</sup></b>
		<b>CIN3+ (N = 96)<sup>c</sup></b>	<b>CIN2+ (N = 176)<sup>c</sup></b>	
<b>HC2 (N = 344)</b>				
PreservCyt	289 (84)	0.98	0.97	0.21
SurePath	269 (78)	0.98	0.96	0.28
Agreement (%)	89.5	95.6	94.5	87.7
Discordant <sup>a</sup>	28 vs 8	1 vs 1	3 vs 2	25 vs 6
P-value <sup>b</sup>	0.001	1	1	0.001
<b>RealTime (N = 630)</b>				
PreservCyt	476 (76)	0.99	0.95	0.32
SurePath	447 (71)	0.97	0.91	0.37
Agreement (%)	93.8	95.8	94.3	93.6
Discordant <sup>a</sup>	34 vs 5	3 vs 1	8 vs 2	26 vs 3
P-value <sup>b</sup>	2.4 x 10 <sup>-6</sup>	0.62	0.11	1.5 x 10 <sup>-5</sup>
<b>Onclarity (N = 630)</b>				
PreservCyt	486 (77)	1.00	0.97	0.31
SurePath	494 (78)	1.00	0.97	0.29
Agreement (%)	97.1	100	100	96
Discordant <sup>a</sup>	5 vs 13	0 vs 0	0 vs 0	5 vs 13
P-value <sup>b</sup>	0.10	1	1	0.10
<b>PapType (N= 585)</b>				
PreservCyt	465 (79)	0.96	0.93	0.26

SurePath	469 (80)	0.96	0.94	0.25
Agreement (%)	93.5	93.4	95.8	92.6
Discordant <sup>a</sup>	17 vs 21	3 vs 3	3 vs 4	14 vs 17
P-value <sup>b</sup>	0.63	1	1	0.72

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**Aptima (N = 613)**

PreservCyt	476 (78)	100	0.98	0.30
SurePath	446 (73)	0.99	0.93	0.35
Agreement (%)	90.2	100	95.4	88.1
Discordant <sup>a</sup>	45 vs 15	0 vs 0	8 vs 0	37 vs 15
P-value <sup>b</sup>	1.3 x 10 <sup>-4</sup>	1	0.01	0.003

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**OncoHealth (N= 630)**

PreservCyt	356 (57)	0.58	0.60	0.45
SurePath	301 (48)	0.55	0.52	0.54
Agreement (%)	55.4	46.9	49.4	57.7
Discordant <sup>a</sup>	168 vs 113	27 vs 24	51 vs 38	117 vs 75
P-value <sup>b</sup>	0.001	0.78	0.203	0.003

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352 <sup>a</sup>PreservCyt+/SurePath- vs SurePath+/PreservCyt-

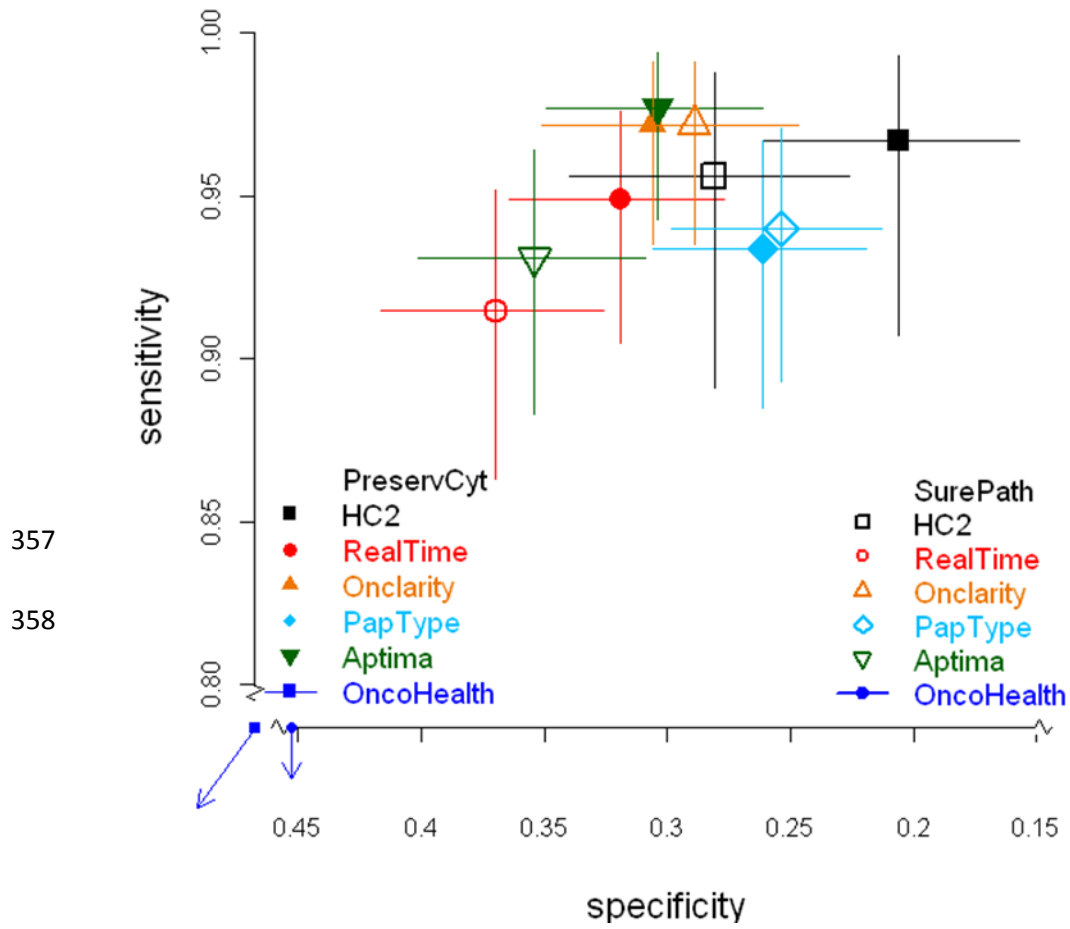
353 <sup>b</sup> McNemar's test

354 <sup>c</sup> Number refers to the whole population of N=630. See Figure 1 for reduced numbers for HC2,

355 PapType and Aptima

356

Figure 2. Sensitivity and specificity for CIN2+ (with 95%CI) by HPV test and transport medium. Solid shapes show PreservCyt and open shapes are for SurePath.



359 **Table 3.**

360 **A) Median signal strength (viral load) by test, transport medium and order of the test for samples**  
 361 **from women positive for at least one medium using the specified test. Units are the ratio to a**  
 362 **reference sample except for RealTime and Onclarity which are CT values. Type specific results for**  
 363 **HPV 16 and 18 (where available) are shown in the lower part of the table.**

364 **B) 2-sided P-values for comparisons between different media and order using unpaired**  
 365 **comparisons by the Wilcoxon RankSum test for samples positive for at least one medium.**

366 **A) Median signal strength (RIU or CT)**

HPV Test	Medium and order of sampling			
	PreservCyt 1 <sup>st</sup>	PreservCyt 2 <sup>nd</sup>	SurePath 1 <sup>st</sup>	SurePath 2 <sup>nd</sup>
HC2	235.03	292.80	90.54	53.08
RealTime	21.30	22.03	23.64	25.85
Onclarity	24.16	24.37	23.16	24.32
PapType	30.53	27.43	25.79	19.54
Aptima	10.67	10.81	10.55	9.80
OncoHealth	1.04	2.10	1.00	1.78
RealTime 16	20.17	22.05	24.22	24.43
RealTime 18	23.09	21.90	23.12	26.91
Onclarity 16	25.16	25.66	24.11	24.75
Onclarity 18	27.42	25.79	25.46	26.63
PapType 16	28.43	32.28	27.83	19.88
PapType 18	13.97	5.76	13.67	11.25

367 **B) Significance levels (2-sided)**

HPV Test	PreservCyt 1 <sup>st</sup>	PreservCyt 1 <sup>st</sup>	SurePath 1 <sup>st</sup>	PreservCyt 2 <sup>nd</sup>
	vs	vs	vs	vs
	PreservCyt 2 <sup>nd</sup>	SurePath 1 <sup>st</sup>	SurePath 2 <sup>nd</sup>	SurePath 2 <sup>nd</sup>
HC2	0.998	0.009	0.011	1.04e-07
RealTime	0.092	1.83 x 10 <sup>-5</sup>	2.2 x 10 <sup>-8</sup>	8.6 x 10 <sup>-15</sup>
Onclarity	0.104	0.033	0.011	0.182
PapType	0.167	0.034	0.004	6.0 x 10 <sup>-4</sup>
Aptima	0.313	0.094	0.012	4.7 x 10 <sup>-7</sup>
OncoHealth	1.44 x 10 <sup>-25</sup>	0.155	3.8 x 10 <sup>-22</sup>	1.2 x 10 <sup>-4</sup>

RealTime 16	0.029	$1.9 \times 10^{-4}$	0.038	$2.0 \times 10^{-5}$
RealTime 18	0.859	0.414	0.024	0.004
Onclarity 16	0.353	0.123	0.208	0.101
Onclarity 18	0.781	0.174	0.314	1.000
PapType 16	0.460	0.810	0.078	0.015
PapType 18	0.857	0.754	0.512	0.967

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368

369 **Table 4. Spearman's  $\rho$  Correlation coefficient and slope when SurePath values are regressed on**  
 370 **PreservCyt values using L1 (robust) regression where values are either the log (1+RIU value) or**  
 371 **(minus) Ct value and sample order is accounted for. (See methods section). One tailed p-values**  
 372 **compare observed slope to unity (no difference in viral load between media).**

HPV Test	N <sup>a</sup>	Spearman's $\rho$ (95% CI)	Slope (95%CI); P-value (vs unity)
HC2	297	0.814 (0.771, 0.849)	0.966 (0.875, 1.057); p=0.231
RealTime	481	0.724 (0.678, 0.764)	0.823 (0.724, 0.923); p=2.5 x 10 <sup>-4</sup>
Onclarity	499	0.884 (0.864, 0.902)	0.841 (0.778, 0.903); p=3.0 x 10 <sup>-7</sup>
PapType	486	0.756 (0.715, 0.792)	0.871 (0.780, 0.963); p= 0.003
Aptima	491	0.683 (0.633, 0.727)	0.676 (0.514, 0.838); p=4.5 x 10 <sup>-5</sup>
OncoHealth	469	-0.133 (-0.221, -0.043)	0.242 (0.121, 0.362); p<2.010 <sup>-16</sup>
RealTime 16	159	0.574 (0.460, 0.670)	0.653 (0.400, 0.906); p= 0.004
RealTime 18	55	0.660 (0.478, 0.787)	0.649 (0.242, 1.056); p=0.046
Onclarity 16	161	0.838 (0.786, 0.879)	0.827 (0.677, 0.977); p= 0.012
Onclarity 18	57	0.890 (0.820, 0.934)	0.833 (0.561, 1.105); p= 0.114
PapType 16	166	0.771 (0.701, 0.826)	0.942 (0.839, 1.046); p=0.137
PapType 18	88	0.748 (0.638, 0.828)	0.914 (0.735, 1.094); p=0.175

373 <sup>a</sup> Positive at least for one test

374

**Supplementary Table S1. Referral smear and worst reviewed histology**

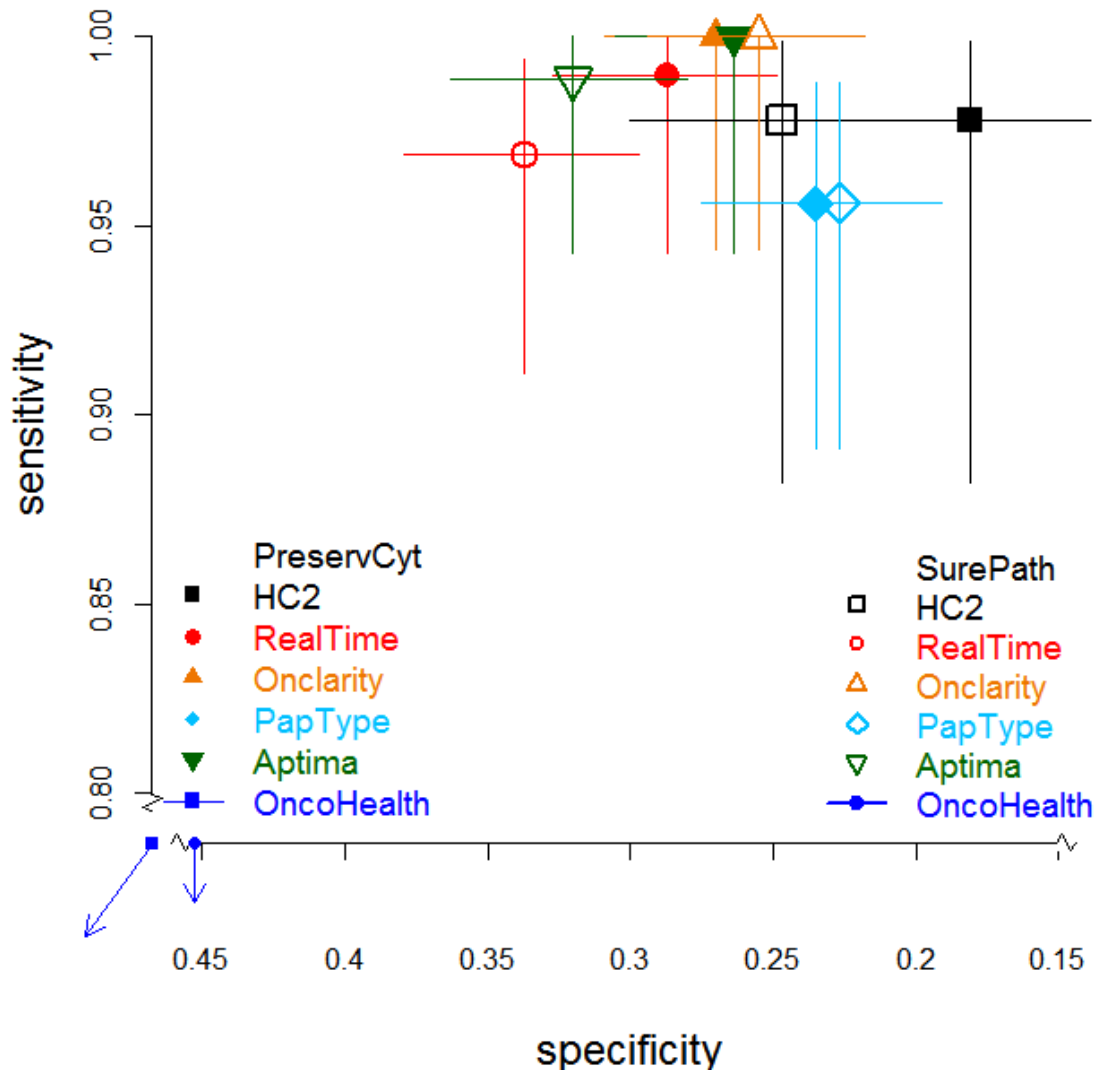
Referral cytology (N)	Worst histology (N)							Total (% N)
	Normal colposcopy no biopsy	Inadequate	Normal	CIN1/HPV only	CIN2	CIN3 or CGIN	Invasive carcinoma	
Borderline dyskaryosis No HPV triage	19	1	69	18	10	13	0	130 (20.6)
Borderline dyskaryosis (HPV +ve)	5	1	27	17	10	3	0	63 (10.0)
Mild dyskaryosis No HPV triage	52	5	134	67	48	32	0	338 (53.7)
Mild dyskaryosis (HPV +ve)	4	0	12	16	4	6	0	42 (6.7)
Moderate dyskaryosis	1	1	1	3	6	25	0	37 (5.9)
Severe dyskaryosis/ glandular	0	0	0	1	2	15	2	20 (3.2)
Total (%N)	81 (12.8)	8 (1.3)	243 (38.6)	122 (19.4)	80 (12.7)	94 (14.9)	2 (0.3)	630 (100.0)

375



376 **Supplementary Figure S1. Sensitivity and Specificity for CIN3+ by HPV test and transport medium.**

377 **Solid shapes show PreservCyt and open shapes are SurePath**

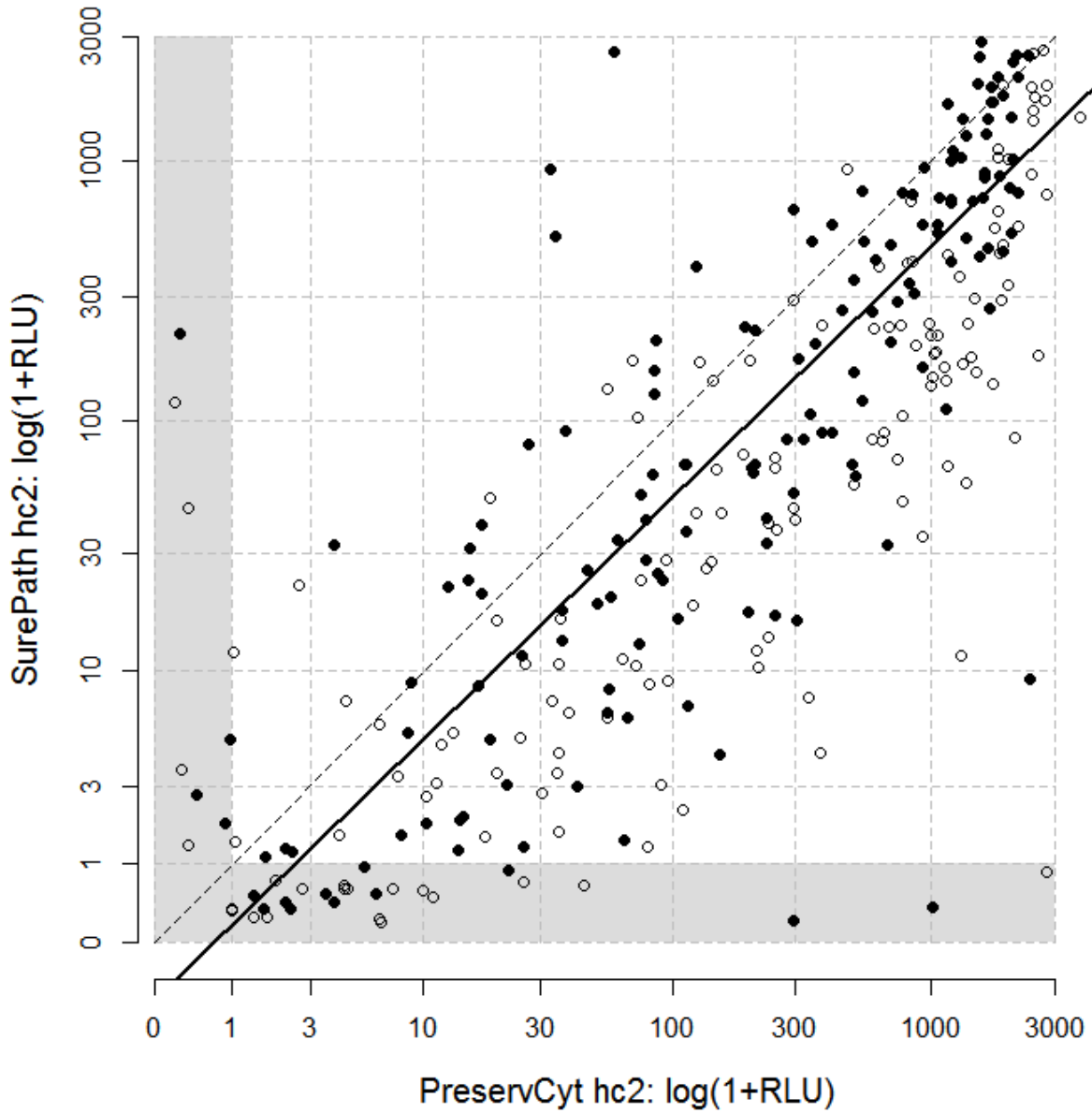


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380 **Supplementary Figure S2. Scatterplot of *digene* HPV Test RLU values for all tested samples. The**  
381 **solid line is the regression line for SurePath regressed on PreservCyt adjusted for sample order.**  
382 **The dashed line is the 45 degree line. Samples with PreservCyt first are open and those with**  
383 **SurePath first are solid. Shaded area indicates values below the positivity cut-off.**

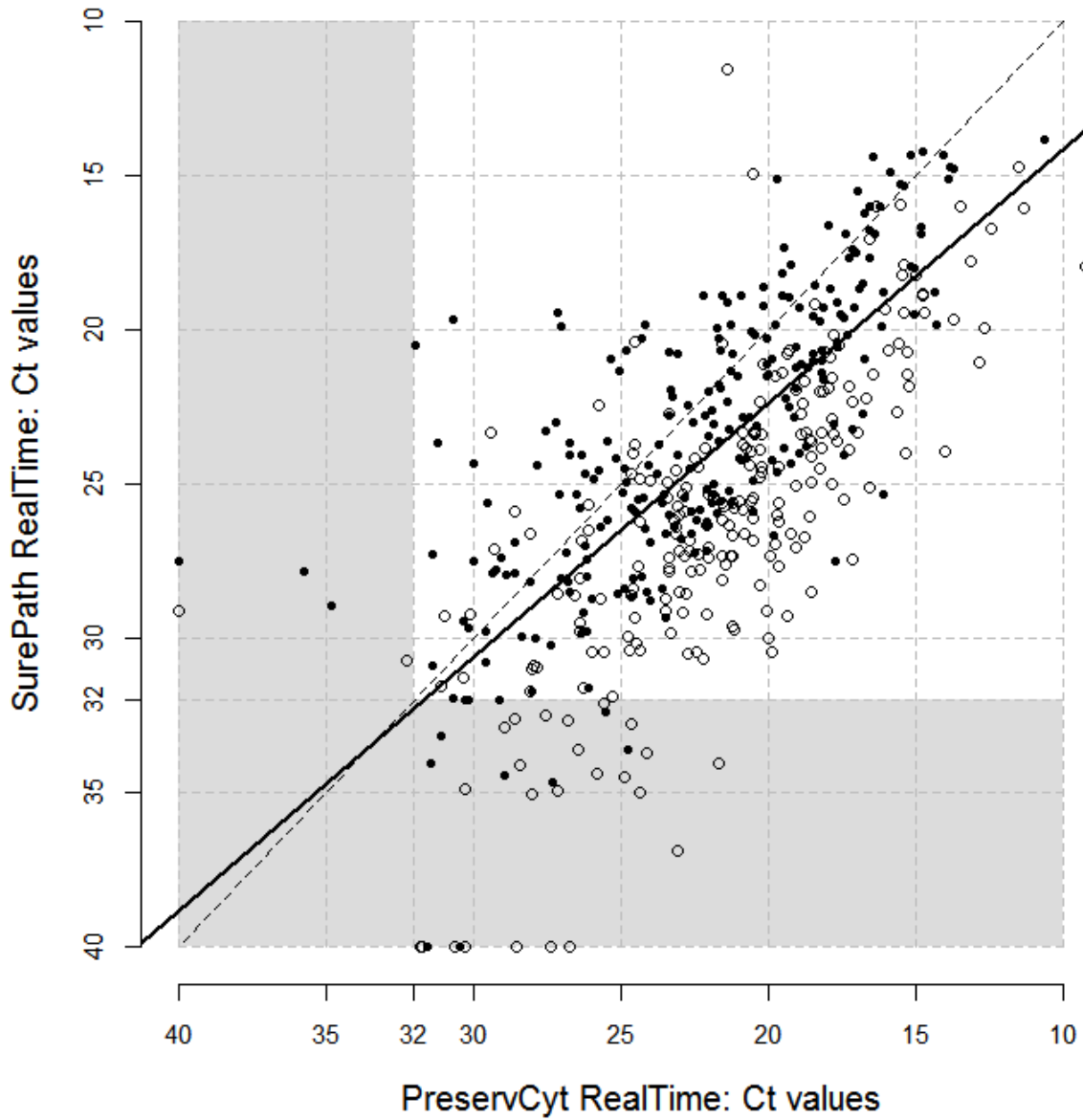
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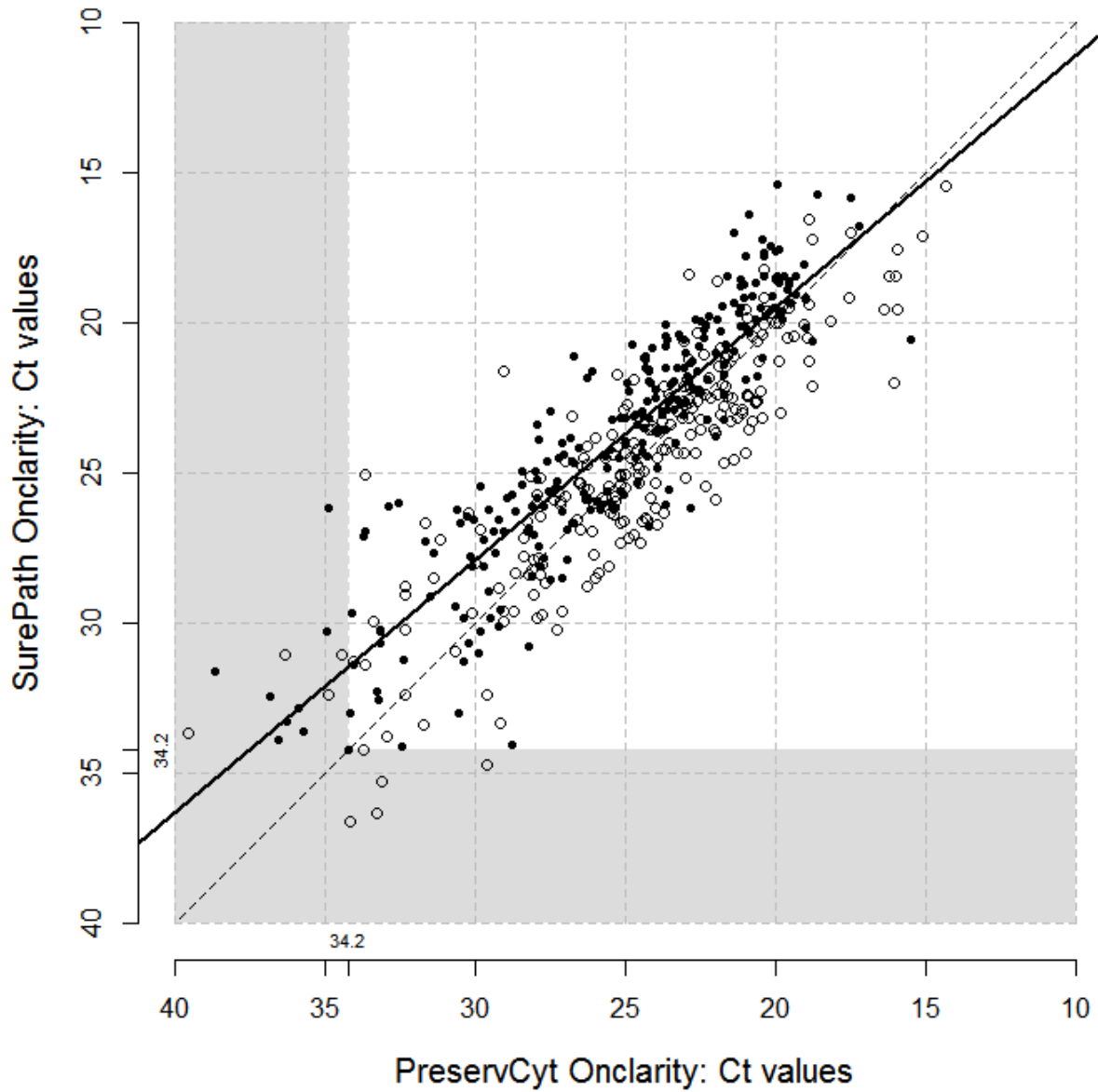
387 **Supplementary Figure S3. Scatterplot of Realtime Ct values for samples that are amplified for at**  
388 **least one test. The solid line is the regression line for SurePath regressed on PreservCyt adjusted**  
389 **for sample order. The dashed line is the 45 degree line. Samples with PreservCyt first are open and**  
390 **those with SurePath first are solid. Shaded area indicates values below the positivity cut-off.**



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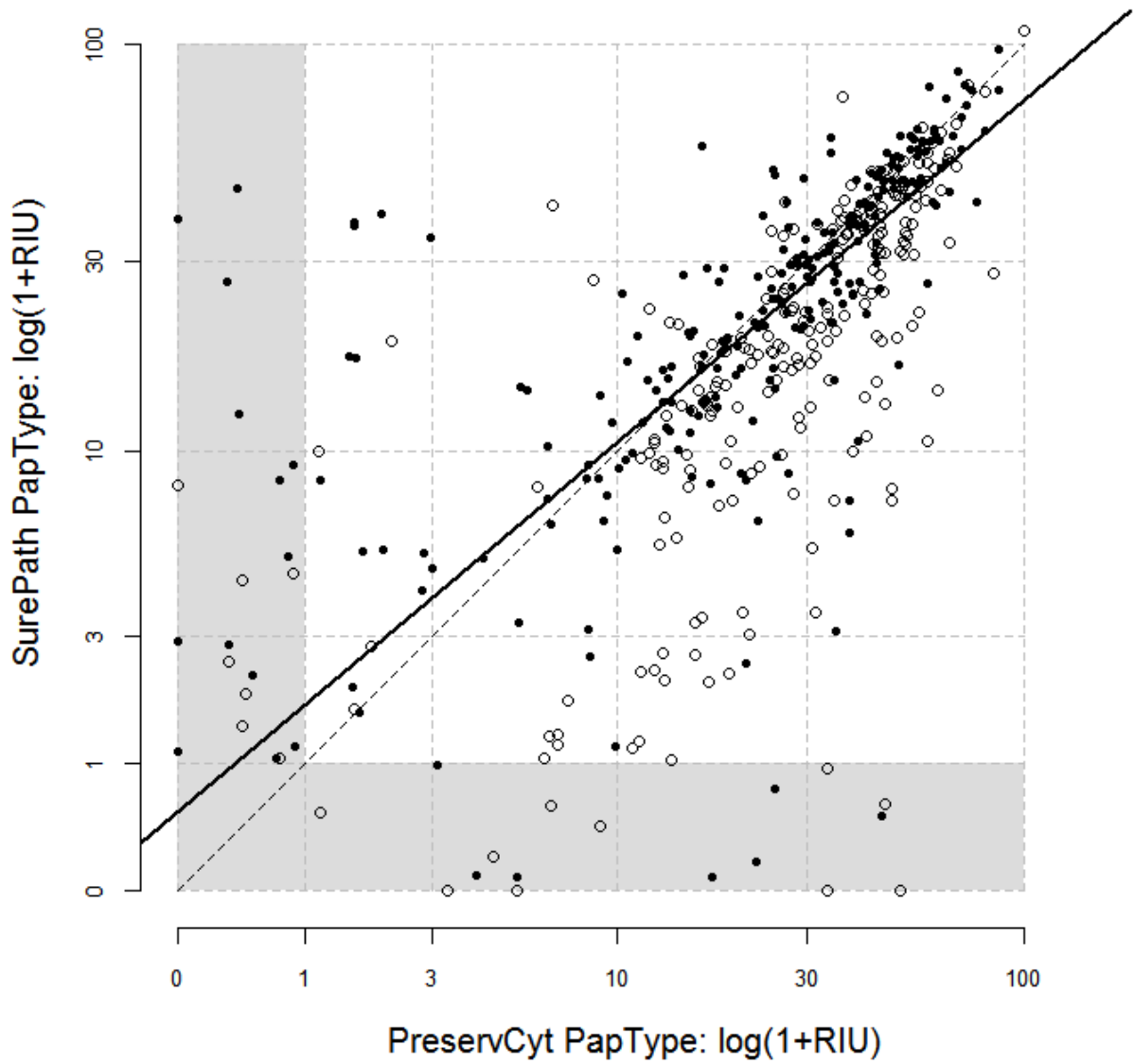
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393 **Supplementary Figure S4. Scatterplot of Onclarity Ct values for samples that are amplified for at**  
394 **least one test. The solid line is the regression line for SurePath regressed on PreservCyt adjusted**  
395 **for sample order. The dashed line is the 45 degree line. Samples with PreservCyt first are open and**  
396 **those with SurePath first are solid. Shaded area indicates values below the positivity cut-off.**



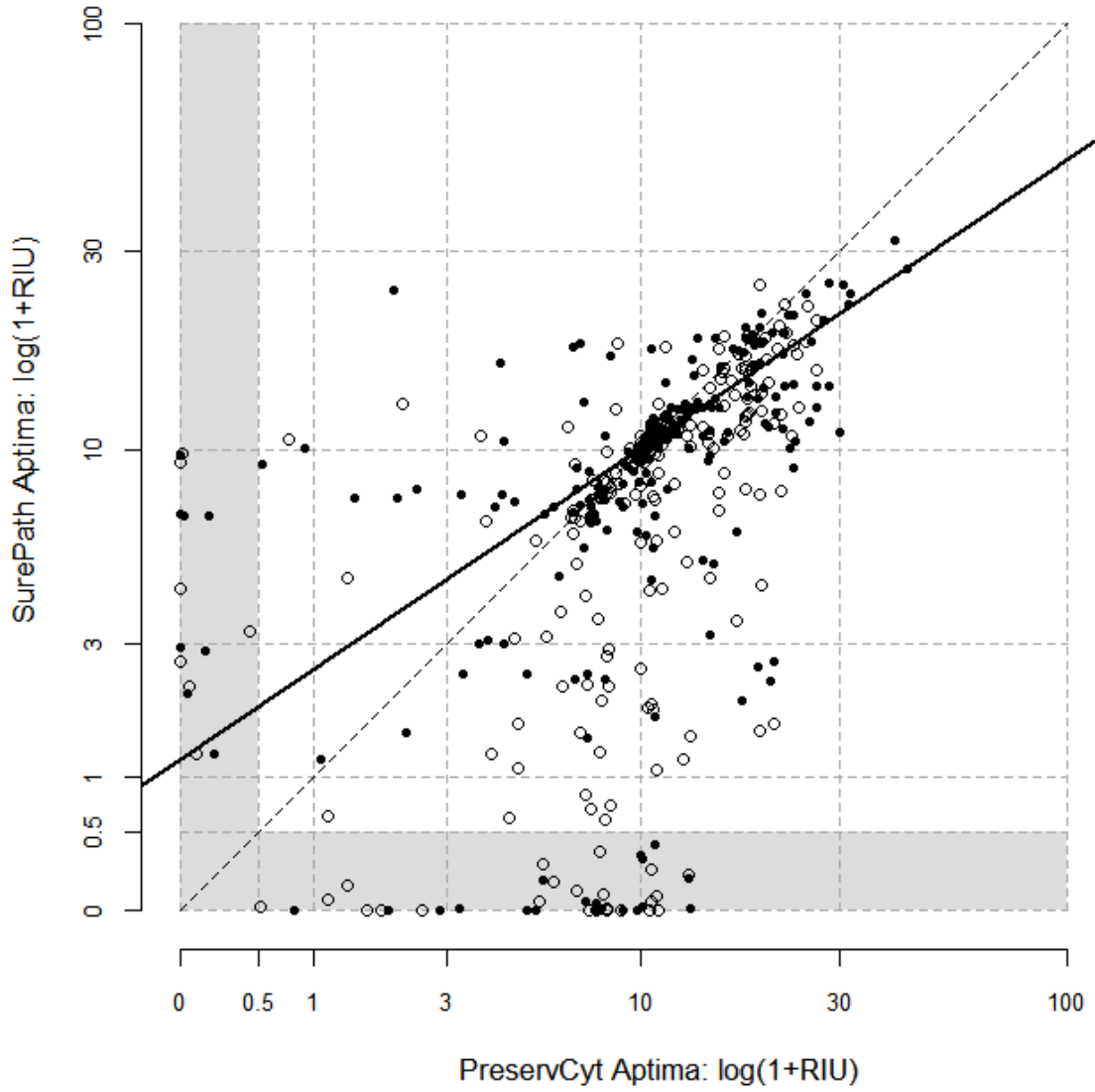
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399 **Supplementary Figure S5. Scatterplot of PapType RIU values. The solid line is the regression line**  
400 **for SurePath regressed on PreservCyt adjusted for sample order. The dashed line is the 45 degree**  
401 **line. Samples with PreservCyt first are open and those with SurePath first are solid. Shaded area**  
402 **indicates values below the positivity cut-off.**



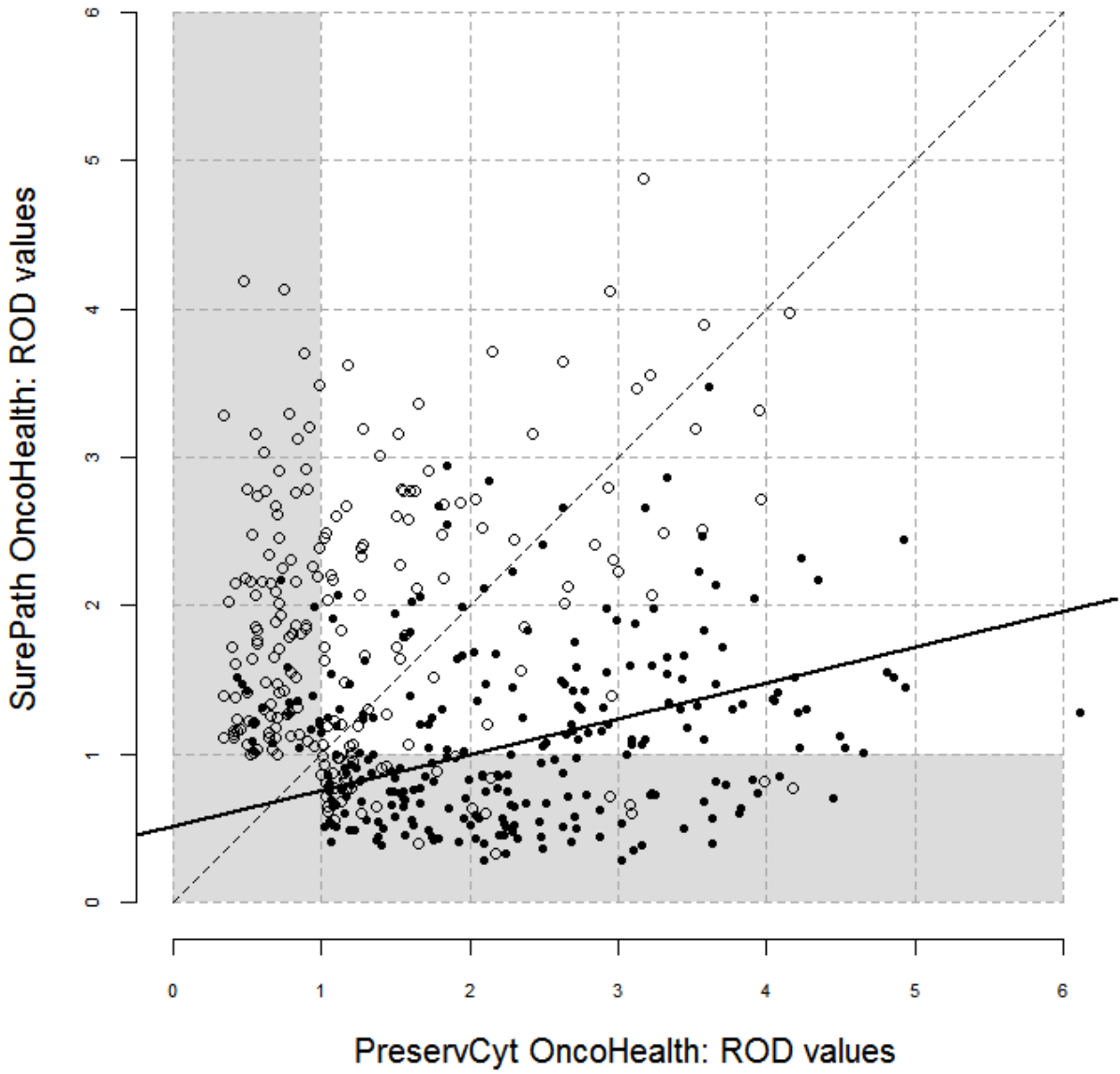
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405 **Supplementary Figure S6. Scatterplot of Aptima RIU values. The solid line is the regression line for**  
406 **SurePath regressed on PreservCyt adjusted for sample order. The dashed line is the 45 degree line.**  
407 **Samples with PreservCyt first are open and those with SurePath first are solid. Shaded area**  
408 **indicates values below the positivity cut-off.**



409  
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411 **Supplementary Figure S7. Scatterplot of OncoHealth ROD values. The solid line is the regression**  
412 **line for SurePath regressed on PreservCyt adjusted for sample order. The dashed line is the 45**  
413 **degree line. Samples with PreservCyt first are open and those with SurePath first are solid. Shaded**  
414 **area indicates values below the positivity cut-off.**



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