

**REPRODUCIBILITY OF THE HPV ONCOTECT 3DX™ ASSAY ON CERVICAL CYTOLOGY SPECIMENS FOR  
DETECTION OF HPV E6, E7 MRNA AND CELL CYCLE PROLIFERATION**

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**Background**

We report the performance of an assay that measures both expression of E6, E7 mRNA and cellular proliferation on a cell by cell basis with residual cervical cytology samples.

**Methods**

Residual de-identified cervical cytology samples in ThinPrep media were used to determine intra-run, inter-operator, inter-system, inter-day, and inter-site reproducibility. Negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) were determined by pairwise comparisons. The inter-run and inter-operator portions of this study, samples were pooled based on HPV DNA status. Samples were split and tested at two sites for the inter-site study: TriCore Reference Laboratories (TRL) and IncellDx. Samples were analyzed on three Beckman Coulter CytoFLEX cytometers, one at TRL and two at IncellDx. Results were compared based on a dual cut-off of  $\geq 4.6\%$  E6, E7 expression and  $\geq 4\%$  Post G1.

Metric	Samples	Sites	Operators	Instruments	Days	Non-Redundant Pairs
Intra-Run	HPV DNA +/- Pools	IncellDx	1	1	8	24
Inter-Operator	HPV DNA +/- Pools	IncellDx	2	1	8	72
Inter-System	Individual Samples	IncellDx	1	2	1	96
Inter-Day	Individual Samples	TRL	1	1	5	240
Inter-Site	Individual Samples	IncellDx and TRL	2	2	1	56

**Results**

Reproducibility Metric	Results (HPV DNA- Pool)	Results (HPV DNA+ Pool)
Intra-Run	NPA = 100% PPA = 100% OA = 100%	NPA = 100% * PPA = NA OA = 100%
Inter-Operator	NPA = 85% * PPA = NA OA = 71%	NPA = 100% * PPA = NA OA = 78%

Reproducibility Metric	Non-pooled Sample Results
Inter-System	NPA = 95% PPA = 50% OA = 92%
Inter-Day	NPA = 92% PPA = 100% OA = 93%
Inter-Site	NPA = 92% * PPA = NA OA = 86%

\*No positive concordant results present

## **Conclusions**

The reproducibility of the HPV OncoTect 3Dx assay is acceptable in most of the reproducibility measurements at the two laboratory sites. Inter-system PPA of 50% is a result of insufficient positive samples in the dataset. There were only 4 samples with concordant positive calls and another 4 samples with discordant calls resulting in Inter-system PPA of 50%. With additional positive samples, it is expected that this value would improve.