Fully Integrated, Automatic, and Rapid Molecular Detection and Identification of 20 Clinically Relevant HPV types using the Rheonix CARD™ Platform

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Abstract

Objective: To rapidly, easily and automatically detect and distinguish at least 20 types of clinically relevant human papilloma virus (HPV) directly from clinical samples on the Rheonix CARD™ (Chemistry and Reagent Device) system.

Relevance: Cervical cancer is the leading cause of cancer-related deaths among women in low-income countries and is the second leading cause of cancer-related deaths for women on a worldwide basis. Among currently FDA-approved molecular diagnostics, none are capable of distinguishing the various HPVs other than to classify them as "high" or "low" risk types. Rheonix CARD™ technology is designed to detect and specifically distinguish 20 HPV types.

Methodology: A vaginal swab is collected in our proprietary transport media which allows for extended room temperature storage, if necessary. An aliquot is applied to the sample reservoir and the run initiated. Without any further intervention by the analyst, all the following steps are automatically performed: cell lysis, nucleic acid purification, PCR amplification and multiplexed end-point detection on a low density microarray.

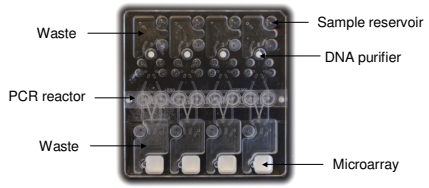
Validation: Analysis of 69 clinical specimens was directly compared on the Rheonix HPV CARD™ test to an FDA-approved product. Of those specimens, 48 were found to be HPV negative by both tests, 14 were determined to be HPV positive by both tests, and 6 specimens, while negative on the comparison test, were positive on the Rheonix HPV CARD™ test. The presence of HPV in the discordant samples was confirmed by amplicon sequencing, thus indicating the accuracy of the Rheonix method.

Rheonix CARD™ Procedure

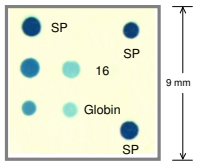
The Rheonix HPV CARD™ test (not yet cleared by FDA for human IVD applications) is capable of performing a fully automated analysis of human cervical samples for the presence of 20 clinically relevant HPV types in a multiplex PCR assay. Briefly described, after the operator introduces the vaginal swab, previously collected and stored in a transfer buffer, into the device (***the only operator step***), the remaining operations are all performed by the Rheonix CARD™.

1. Cells are lysed and DNA is purified.
2. DNA is subjected to multiplex PCR in the presence of biotinylated primers resulting in amplification of any one of the 20 target HPV types and the human β-globin gene.
3. HPV and globin amplicons are denatured and delivered onto the DNA microarray reactor followed by hybridization to the specific capture probes.
4. Hybridized strands are detected via incubation with streptavidin conjugated HRP and substrate (TMB).
5. Image analysis identifies the specific HPV type(s).

Rheonix HPV CARD™



Representative Results



Filter Key

SP	51	33	56	39	SP
45	31	52	35	58	59
16-H	66	16-M	PC2	16-L	PC3
18-L	PC1	18-M	68	18-M	53
Gb-H	11	Gb-M	43	Gb-L	PC1
6	PC2	42	PC3	44	SP

The above filter image resulted following processing of clinical sample # 9030 (see Table on the right) on the Rheonix HPV CARD™. The three corner spots labeled with "SP" are spotting controls; the two spots in the second row show the positive HPV 16 result; and the third row shows detection of globin used as an internal control indicating successful nucleic acid purification. All probes are spotted at the same concentration except HPV 16, 18 and globin, which are spotted at 3 different concentrations.

Comparison against an FDA-approved product

Evaluation of samples that were negative on an FDA-approved product

Sample #	FDA-approved Product	Rheonix HPV CARD™ Results
Negative on Both Tests	N = 48	N/A
9040	0.12 (negative)	18
9032	0.16 (negative)	18
9014	0.20 (negative)	58
9053	0.28 (negative)	66
9029	0.48 (negative)	51, 42
9055	0.50 (negative)	31

Evaluation of samples that were positive on an FDA-approved product

Sample #	FDA-approved Product	Rheonix HPV CARD™ Results
9015	1.01	None
9024	1.07	42
9054	2.44	18, 33
9050	2.47	56
8992	5.45	33
9047	11.40	16
9016	11.40	56, 58
8987	15.70	39
9003	20.20	18
8988	92.70	53
9004	179.00	31, 56, 16
9011	222.00	66
8991	1008.00	66, 58
9025	1311.00	18
9030	1472.00	16

Conclusions

The Rheonix HPV CARD™ test provides a fully automated system for the rapid and reliable molecular detection of 20 clinically relevant HPV types. Furthermore, due to the low cost and minimum training required in using the Rheonix CARD™ product, this test will have widespread application potentials in both industrialized and developing nations.

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