Development of an external run control for HPV primary screening

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INTRODUCTION

- Human Papilloma Virus (HPV) has been shown to be associated with 99.7% of cervical cancers.
- Molecular detection of high risk HPV genotypes (e.g., 16, 18) is increasingly being introduced for cervical cancer screening.
- External quality control (EQC) reagents are needed to monitor performance of kit lots, competence of staff and performance of testing platform.
- A single EQC reagent be developed that assures all high risk HPV genotypes and DNA and mRNA assays?
- Pilot study demonstrated that CaSki cells mixed with MRC-5 cells could perform as HPV 16 control. What about other high risk genotypes? (Figure 1)

RESULTS

- All assays produced positive results for HPV 16 and 18 samples containing at least 10^3 IU/ml (Figure 2).
- Five laboratories provided Ct results for an internal cellular control (Figure 3). All tests recorded a ΔCt above cut-off thus validating the panel.
- The higher Ct values for transduced A549 cells and the differences observed between dilutions of the IS could be test-specific, a result of background genomic DNA or a consequence of poor resuspension.
- All laboratories detected HPV DNA in the three cell-based preparations validating the use of transduced cells for genotypes without where cell lines or sufficient clinical material do not exist for formulating run controls.
- The reproducibility of assay performance provided by individual labs was high with the highest standard deviation of 0.99 Ct.
- Inter-laboratory comparison showed consistent testing. Two laboratories (Labs 3 and 4) used Assay 1 with a maximum standard deviation of 1 Ct. Two laboratories (Labs 6 & 7) used Assay 4 with a maximum standard deviation of 1.38 Ct.

CONCLUSIONS

- All HPV assays that were tested could detect HPV 16 & 18 DNA positive run control samples. The mRNA test detected HPV 16 but not 18.
- Challenge of cell count in multiplex could be mitigated by selecting higher copy number transduced cell lines.
- A common run control to test positive with all HPV DNA tests will require a HPV 16/18 concentration between 6.25 x 10^3 and 6.25 x 10^4 IU/ml.
- Results prove the principle of creating HPV/lentiviral transduced cell line to create common EQC for DNA and mRNA based molecular screen for HPV
- Overall, this study has produced universal run control materials for HPV 16 and 18 that can be resuspended in ThinPrep or SurePath without affecting performance.

CURRENT WORK

Construction of lentiviral transduced cells for remaining high risk HPV types present in molecular screening assay.
Keen to identify groups willing to evaluate these materials in future collaborative studies.

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