High throughput HPV screening using a one step multiplex real-time PCR based system

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Abstract

Primary HPV screening is an emerging concept, which may fundamentally influence cervical cancer screening (J. Cuzick et al. Vaccine 26S (2008) K29–K41). To fulfill the demand we have developed a molecular beacon based one step multiplex real-time PCR (MB-RT PCR) system that is designed for the high throughput detection of 14 high-risk HPVs (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) on the ABI7900HT instrument, in 96 or 384 well plate format. Detection is achieved in 3 dye channels. Recognizing the demand for the identification of HPV vaccine types, HPV 16 and 18 are detected together in one channel. The other high-risk types are detected in groups separately and an artificial internal control is also detected to control for false negative reactions.

Using two patient populations; a cervical screening population and a colposcopy referral population, the performance of the MB-RT PCR was compared to cytology, histology, Full Spectrum HPV Amplification and Detection System (GenoID, IVD-CE mark), Amplicor HPV Test (Roche) and Hybrid Capture II (hc2, Qiagen). We propose the MB-RT PCR is a useful tool for high throughput HPV based cervical screening. In addition this technology has the capability for semiquantitative viral load determination which could potentially be used to improve specificity of primary HPV screening.

Materials & Methods

Patient population. The technique proposed here was evaluated in 3 patient populations; (1) cohort of a cervical screening population (n=149, selected based on hc2 positivity), (2) cohort of a cervical screening population (n=180) both recruited through CERVIVA The Irish Cervical Screening Research consortium, (3) a population of women attending colposcopy in Jedlik Ányos HPV_SCREEN multicentric clinical study in Hungary (n=293). HPV testing was performed on residual PreservCyt cervical smear specimens after cytological diagnosis was made.

HPV Detection Technologies. Four approaches were taken for HPV detection; High Risk hc2 Assay (Qiagen), Amplicor HPV test (Roche), Full spectrum HPV Amplification and Detection System (GenoID) and the MB RT PCR test (GenoID). All assays were performed according to manufacturer's instructions. HPV copy number was determined by type specific TaqMan reactions with a following setup of 15 µl mastermix and 10 µl DNA sample including specific primer pair, 200 nM type specific probe and 1 U ITaq (BioRad). PCR reactions were performed on ABI StepOnePlus machine with the thermoprofile 1x(2min.@ 90°C), 45x(10sec@95°C; 30sec@60°C). Human DNA amount was determined by TaqMan based factorV reaction with the reaction setup of 15 µl mastermix and 10 µl DNA sample including, 1xPCR Buffer (Applied Biosystems, Foster City, CA), 4mM MgCl2, 250 µM dNTP each, 800 nM primer pair 200 nM probe 1 U AmpliTaqGold (Applied Biosystems, Foster City, CA). PCR reactions were performed on ABI StepOnePlus machine with the thermoprofile of 1x(10min@ 90°C), 45x(10sec@95°C; 30sec@60°C) detection was done at 60°C. Evaluation of dataset was done according to basic contingency statistics.

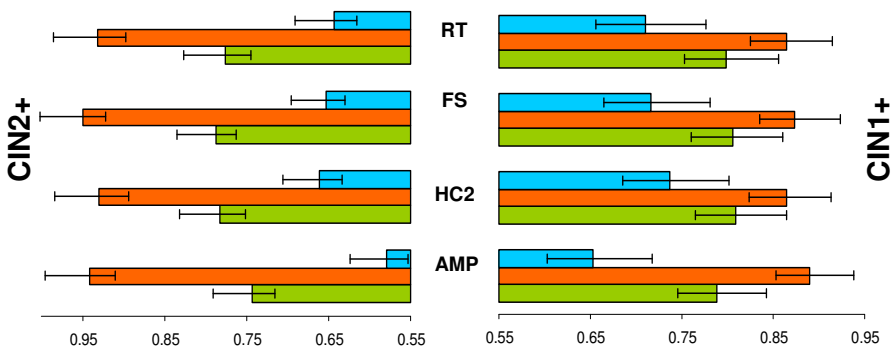

Figure 3. Evaluation of clinical performance of different HPV detection tests compared to CIN1+ and CIN2+ histology using population (2)
MB-RT PCR (RT), Full Spectrum HPV Amplification and Detection System (FS), Hybrid Capture II (HC2) and Amplicor HPV Test (AMP) tests were compared to CIN1+ and CIN2+ histology. No significant difference have been found, except for Amplicor HPV Test, where CIN2+ specificity were lower than the others. Note the especially close agreement of MB-RT PCR and Hybrid Capture II tests. Accuracy - green, sensitivity - orange and specificity - blue.

Discussion

The MB-Real Time PCR technology clearly offers less technological barriers for the introduction of primary HPV screening protocols than the conventional techniques. Using two populations; a colposcopic referral population and a cervical screening population, the MB-RT PCR real-time PCR test shows equivalency to other HPV tests including the hc2, which was recently suggested as the gold standard HPV test for clinical evaluation (Meijer CJ. Int J Cancer. 2008 Sep 19.). The MB-RT PCR test is amenable for robotic automation and further evalution on a screening population is ongoing.

Controversially, viral load determination has been proposed as an adjunct to conventional HPV testing, to improve the specificity of HPV assays, but its clinical performance has not been established definitively. However, using a more robust genomic DNA normalized viral load method such as that described here, has a new potential to stratify a HPV positive however low disease prevalance population in the primary HPV screening population, and could ultimately improve primary HPV cervical screening and reduce cytology/colposcopic referral rates. Our results based on colposcopic referral population is a low estimate of the potential benefits of such approach, larger screening studies need to verify the effect.

Acknowledgements

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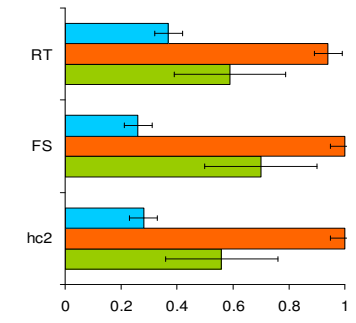

Figure 1. Comparison of HPV tests for the detection in a cervical screening population (population 2) (n=180). MB-RT PCR (RT), Full Spectrum HPV Amplification and Detection System (FS) and Hybrid Capture II (hc2) were compared for the detection of cytology. Accuracy - green, sensitivity - orange and specificity - blue. (Age range 18-69, median age 31).

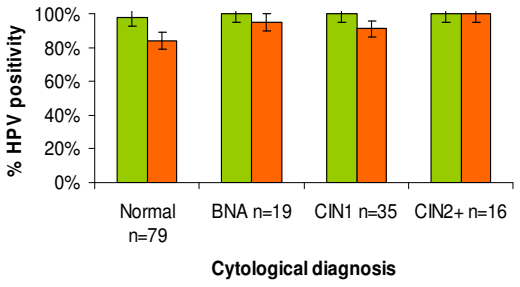

Figure 2. Comparison of different HPV detection tests in a cervical screening population of women (n=149 specimens selected which were positive for HPV DNA by hc2). Specimens representing a range of cytological disease categories (normal – CIN3), which were positive for HPV (by hc2) were tested for HPV by Full Spectrum HPV Amplification and Detection System (FS) green and MB-RT PCR (RT) orange. Note 100% detection of HPV by both tests in CIN2+ cytology.

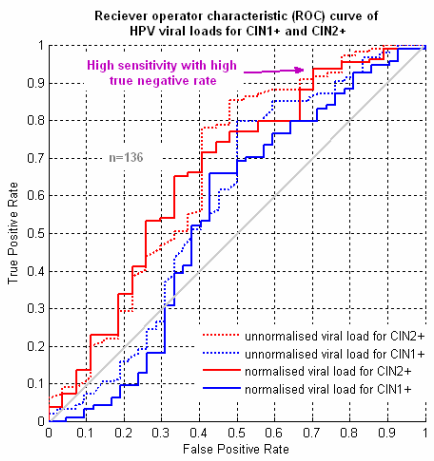

Figure 4. Receiver operator characteristic (ROC) curve of normalized and unnormalized MB-RT PCR viral load data for CIN1+ and CIN2+ targets
Semiquantitative viral load data were determined for colposcopic referral population (n=136 HPV positive cases). The viral load values were normalized for the genomic DNA copy number of the samples (continuous lines) and compared to unnormalized data (dotted lines) both for CIN1+ (blue) and CIN2+ (red) targets. Especially normalized CIN2+ curve shows high true negative rate (30%) at 94% sensitivity. This could be used to stratify a HPV positive but low histology positive population.