Abstract No: 1683

Presentation at ESCV 2015: Poster 1
Frequent detection of human papillomavirus DNA in oral lesions

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Background: Human papillomavirus (HPV) is estimated to be the cause of 40–80% of oropharyngeal squamous cell carcinoma (OSCC). The incidence of OSCC has significantly increased in the last decade and the prevalence of oral HPV infection in the general population augmented as well. Therefore, there is a need to evaluate the effectiveness of HPV detection for OSCC prevention, using methods less invasive than surgical biopsy. We sought to detect HPV DNA in oral brushings from oral potentially malignant disorders (OPMD) and oral carcinomas. HPV DNA positivity, viral loads and the HPV16 integrational status were evaluated in relation to clinical data.

Methods: A total of 134 individuals attending Odontostomatologic Clinics were enrolled: 77 patients, affected by benign oral lesions, OPMD, or oral carcinoma, and 57 controls with no lesion in the oral mucosa. Oral cells were collected using Cytobrush, a not invasive but site-specific method. HPV DNA was detected with quantitative real-time PCR (qPCR) for the more common low-risk (HPV 6, 11) and high-risk (16, 18, 31, 33, 53, 58) genotypes.

Results: In patients group, 40/77 (51.9%) samples were HPV positive, a rate significantly higher than that found in the controls (33.3%). The percentage of high-risk HPVs and HPV-DNA loads in samples from cases were significantly higher than in controls; among lesions, lichen planus had the highest HPV positivity rate (71.4%), hairy leukoplakia the lowest (33.3%). Interestingly, HPV16 resulted the most frequent genotype in all types of lesions but was integrated only in 3 out of 4 HPV- positive carcinomas and in 3/14 HPV-positive lichen planus.

Conclusion: This study implemented a non-invasive sample collection method with a sensitive and quantitative molecular method, detecting high rates of oral HPV-DNA, particularly in lichen planus, and high integration rates in HPV-positive carcinomas. The fact that HPV oral infection may be common suggest the utility of viral load and integrational status determination to discriminate those lesions at higher risk of malignant progression.

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Development of a new detection tool by real time PCR for the detection of Middle East Respiratory Syndrome human Coronavirus (MERS-HCoV) combining specific primers, probe and a RNA internal control ready to use premix

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Background: Since the emergence of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012 in the Arabian Peninsula, many questions remain on modes of transmission and sources of virus. The MERS-HCoV causes severe respiratory illness. The epidemic origins are uncertain but it is probably a zoonosis. The reservoirs could be the bat or the camel. In 2015-04-29, the MERS-HCoV is responsible of 1110 laboratory confirmed cases and 442 deaths (WHO). In outbreak situations, especially with emerging organisms causing severe human diseases, it is important to develop quickly a test to detect the virus involved. bioMérieux developed a real time PCR assay for the rapid detection of MERS-HCoV combining specific primers, probe and a RNA internal control ready-to-use premix. The results of the analytical sensitivity, the precision determination and the exclusivity study are presented.

Methods: A set of primers and probe (Mers-hCoV primers r-gene® #20-010 and Mers-hCoV probe r-gene® #20-011, bioMérieux) were designed on the S gene, coding for the spike structural protein. This set was evaluated in combination with a ready-to-use premix for RNA internal control to constitute a duplex RT PCR assay. The internal control, added before the extraction step, allows to check simultaneously extraction efficiency and presence of inhibitors. Extractions were performed on NucliSENS® easyMAG® (bioMérieux) followed by amplifications on 7500 Fast Real-Time PCR System (Applied Biosystems®). The analytical sensitivity was performed on 20 replicates of a wide dilution range of In Vitro transcripts. The precision determination was determined on 18 replicates of 3 points corresponding at 40, 20 and 8 times LoD 95%. The exclusivity study was carried out on major human respiratory viruses.

Results: The analytical sensitivity was determined by Probit analysis (MiniTab). The LoD (95% hit rate detection) was 2.89 log copies/mL of In Vitro transcripts. The coefficients of variation obtained in the precision test were ranged between 1.28% and 2.26% for the MERS-HCoV and between 1.95% and 2.39% for the internal control. The exclusivity study was performed with the major human respiratory viruses including other human coronaviruses, and no cross-reaction was observed.

Conclusion: The high quality in analytical sensitivity (2.89) and robustness (1.28–2.39%) were demonstrated. The combination of the MersCoV primers and probe in association with the RNA internal control ready-to-use premix provides a useful tool for the detection of MERS-HCoV virus.

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Acute flaccid paralysis surveillance system in Norway detected two cases of enterovirus D68 infection

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Background: WHO recommends surveillance of patients with acute flaccid paralysis (AFP) to ensure the eradication of wild poliovirus. In Norway the AFP surveillance is used as the gold standard for polio surveillance, with supplementary enterovirus surveillance. Here we describe the Norwegian AFP surveillance system, as well as its performance by assessing the frequency of AFP