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Paper Poster Session
Respiratory virus diagnostics

Performance improvement of influenza A/H1N1pdm09 complete genome massive sequencing by setting a cycle threshold cut-off in monoplex PCR

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Background: Because of the increasing emergence of massive sequencing for diagnosis and research on diseases caused by infectious agents such as influenza, optimization of these processes is needed to reduce the cost of each test and to obtain the greater scientific and economic benefits. The aim of this study is to determine the minimum relative viral load that should have a respiratory sample positive for A/H1N1pdm09 influenza virus to obtain the best performance in massive sequencing.

Material/methods: A prospective and observational analysis was done recruiting 75 respiratory upper and lower respiratory samples from patients diagnosed for A/H1N1pdm09 flu virus during 2013-14 and 2014-15 influenza epidemics. Genetic material was extracted by an *EasyMag Automatic Extracto* (*Biomerieux*) and a Cycle threshold (Ct) viral load semi-quantification was done amplifying the HA of each sample by a real time RT-PCR using *ABI 7500-Fast* platform (*Applied BioSystems*) and influenza CDC subtyping reagents (IRR reference FR-929).For testing the quality of samples previously to sequencing, it was done a manual RNA extraction of each sample using *MagMaxTM-96ViralRNA* reagents (*Life Technologies*), and amplicones of each gene were obtained by means of a RT-PCR with PathAmp™ FluA reagents using an Verifi DX 96 platform (*Life Technologies*). The quality cut-off point of samples was established doing agarose 2% gels to test the

integrity of the eight genes of influenza virus, defining the following categories: Correct (8 fragments); Medium (less than 8 fragments); Degrade (without apparent bands). Massive Sequencing was done using *Ion Torrent PGM* (*Life Technologies*). Acceptation intervals were designed using the average Ct values of samples.

Results: Ct average of samples that showed Correct gels was 27.1 (Max:35.9; Min:18.3), 34.6 for Medium gels (Max:39.5; Min:30.5) and 38.6 for Degraded (Max:41.1; Min:34.8), resulting significant different between these three groups (Student-T; $p<0.01$). It was observed nucleotide gaps and poliNs in some sequences of samples that obtained Medium gels with Cts higher than 36.0, and in some of theme it was not possible to sequence PB1, PB2 and PA genes. All samples with Correct and Medium gels with Cts lower than 36.0 were well sequenced. With these results, Ct 36.0 was established as the cut-off for sequencing procedure. This cut-off was later tested in 21 new samples obtaining Correct gels in all of theme, with high quality of all sequences obtained.

Conclusions: Implementation of a minimum relative viral load of influenza virus can increase the performance of massive sequencing techniques reducing the cost of thereof. To obtain the maximum efficiency of sequencing influenza with Ion Torrent is necessary a Ct of at least 36.0 for obtaining complete and high quality sequences, so it is not recommended to include in these runs clinical samples with relative viral loads lower than this value.