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Evaluation of a new Medium for Molecular Detection of Influenza A & B Viruses in Clinical Specimens

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INTRODUCTION and PURPOSE

Influenza epidemics occur every winter in temperate countries but the epidemiology and the severity of the outbreak varies widely according to the circulating viruses. Laboratory diagnosis is an important tool for the control of the outbreak and the adaptation of health care services in hospitals. In France, the influenza surveillance in the general population relies on the "Réseau Unique" network. The general practitioners or paediatricians collected clinical specimens from patients presenting with influenza like infection. The sampling consists of nasal-nasopharyngeal swab taken within 36 hours after the onset of the disease. The clinical samples collected during outpatient consultation are sent by post. Upon reception at The National Influenza Centre each clinical sample is tested for the detection of influenza viruses by real time RT-PCR and inoculated on MDCK cells for virus isolation and characterization. PCR is now the first choice laboratory test for influenza infection either for diagnostic or the virological surveillance. The molecular technique is rapid, specific and sensitive while cell culture requires the appropriate cell line and an average of several days for virus isolation, making them of limited use for the clinician.
The purpose of this study was planned to assess the performance of a new collection medium for molecular analysis.

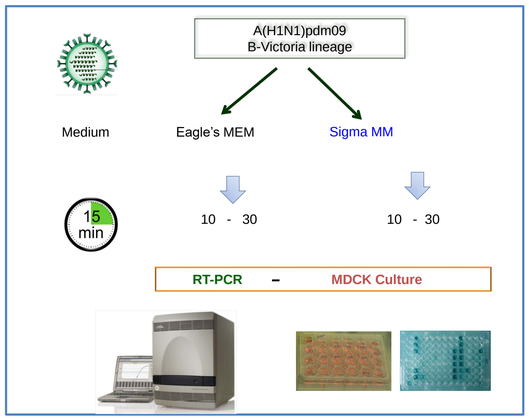
MATERIAL and METHOD

Clinical samples from surveillance in the general population were selected for this evaluation. The choice was made on positive specimens for A(H1N1)pdm09 and B-Victoria lineage both detected by RT-PCR on the original material and for which the virus was isolated on cell culture. The protocol also included the testing of MWE's Sigma Molecular Medium (Sigma MM) for 2 contact times: 10 and 30 minutes. All the tests of this device will be carried out in parallel with the classical medium used in the laboratory (Eagle's MEM).
For an aliquot of 200µl of clinical sample the same volume of Sigma MM or Eagle MEM was added and stored at ambient temperature for 10 and 30 min before testing. Each experimental sample was tested according to the protocols for influenza diagnostic and virus isolation used for the clinical sample from the general population surveillance.
The RT-PCR diagnostic was an in-house one step real-time RT-PCR targeting the M gene for Influenza A and NS gene for Influenza B. Identical experimental samples were inoculated on MDCK for the control of infectivity at the various conditions. The viral growth was tested for cytopathic effect and confirmed by ELISA for influenza A & B virus on the supernatant after 2 days incubation at 33°C. All negative samples were seeded on fresh MDCK cells. After the second passage the presence of the virus was monitored by ELISA after 6 days of cell culture. The absence of detection from the second passage is considered as negative cell grown.

RESULTS - INFECTIVITY MDCK cell CULTURE

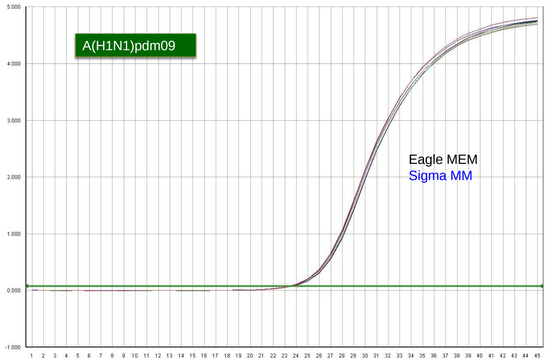
The influenza A(H1N1)pdm09 or B-Victoria lineage were inoculated on 24 well plates in the presence of trypsin. The viruses could be isolated on the first MDCK cells passage with Eagle MEM either by cytopathic effect and ELISA antigen detection in the supernatant. There was no viral growth with Sigma MM even for the short time contact of 10 min. A second passage on fresh cells did not yield any viral growth for either virus, indicating that all infectivity had been eliminated.

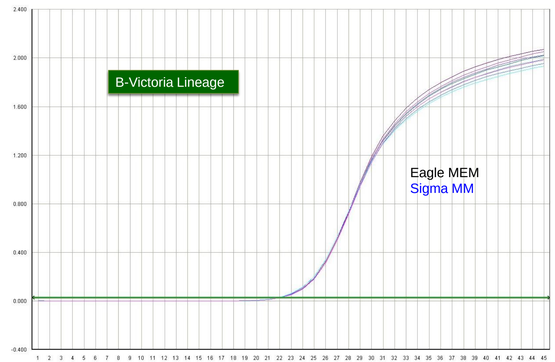
PROTOCOL



RESULTS – RT-PCR

The graph represents the RT-PCR results for A(H1N1)pdm09 and B-Victoria lineage viruses tested in Eagle's MEM or in Sigma MM under the different experimental conditions. The RT-PCR results for the tested Sigma MM were exactly identical to the currently used medium Eagle's MEM





DISCUSSION

In France, the influenza surveillance in the general population is established on RT-PCR detection and MDCK cell inoculation on samples collected by the doctor and sent to the laboratory by post. In this study a new medium (Sigma MM) for safe transportation and molecular detection of microorganisms was evaluated and the performance compared with that of clinical specimens tested by current techniques.
The Sigma MM device showed the same performance for influenza A&B molecular diagnostic as eagle MEM. The RT-PCR results were similar in terms of sensitivity (detection level, Ct values) and efficacy (Fluorescence levels). The data were comparable for Influenza A(H1N1)pdm09 and B-Victoria lineage viruses.
The loss of infectivity was observed for both influenza viruses A&B on cell culture. The device is designed to kill microorganisms but preserve release of the nucleic acids. These results provide evidence that the new swab collection device Sigma MM is reliable for molecular analysis when compared to current Eagle's medium.

The RT-PCR laboratory detection technique is essential for influenza diagnostic because of the continuous evolution and the emergence of new viruses. So the Sigma Molecular Medium should be suitable for influenza A&B molecular diagnostic. This safe device could be an advantage for the shipment of non-infectious viruses/samples, as highly pathogenic species for example, to be used as positives for molecular diagnostic.

Conclusion : From this study, it has been shown that Sigma MM renders the clinical samples non-infectious and safe for handling and transport, while also allowing high quality extraction of nucleic acid for use in RT-PCR. Sigma MM could be used for the molecular diagnostic of clinical specimens for Influenza viruses.

REFERENCES

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