Development of a real-time quantitative multiplex TaqMan-based RT-PCR assay for detection and quantification of measles, mumps and rubella viruses

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Background

Vaccine potency is a major property to assess the quality of live virus vaccines.

Potency assay of trivalent measles, mumps and rubella (MMR) live virus attenuated vaccine is routinely performed by plaque or conventional 50% cell culture infective dose assays. However, these assays are:

- time-consuming,
- labor-intensive,
- typically highly variable,
- dependent on external conditions,
- susceptible to bacterial contamination,
- requiring the availability of highly specific neutralizing antibodies for each of the components in order to allow specific detection of the infectivity of individual components in multivalent vaccines.

Objective

To develop novel methods to estimate the potency of MMR viruses in virus-containing bulks using real-time quantitative PCR for each virus.

Materials and Methods

- **Cell cultures:**
  - continuous cells – Vero, BHK, RK-13
  - semi-continuous cells – Human embryonic fibroblasts, MRC-5 and M-29
- **Virus vaccine strains:**
  - Wistar RA 27/3 Rubella strain
  - L-16 Measles strain
  - L-3 Mumps strain
  - Human Rhinovirus 16 type
- **Laboratory Reference Samples:**
  - Wistar RA 27/3 Rubella virus, 6,3 $lgCCID_{50}$/ml
  - L-16 Measles virus, 5,4 $lgCCID_{50}$/ml
  - L-3 Mumps virus, 7,0 $lgCCID_{50}$/ml
- **International Reference Reagents of MMR vaccines** (Live, NIBSC, UK)
- **Measles-Mumps live attenuated vaccine (Microgen, Russia)**
- **MMR live attenuated vaccine – Priorix** (GlaxoSmithkline Biologicals SA, Belgium)
- **Conventional 50% cell culture infective dose assay ($CCID_{50}$)**
- **Real-time quantitative TaqMan-based reverse-transcription PCR (qPCR-RT)**

Results

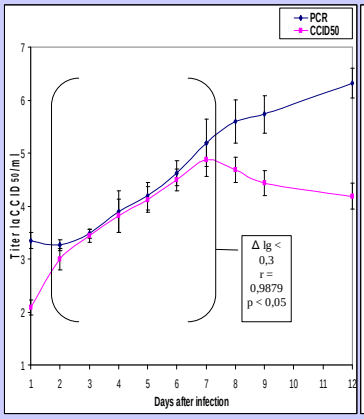

Fig.1. Growth curve for rubella virus as measured by qPCR-RT and $CCID_{50}$.

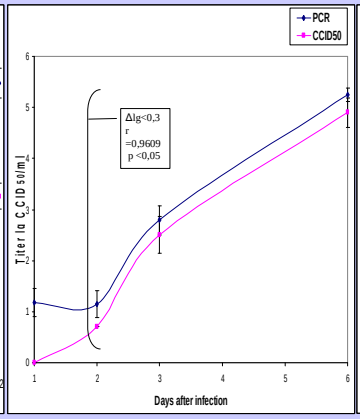

Fig.2. Growth curve for measles virus as measured by qPCR-RT and $CCID_{50}$.

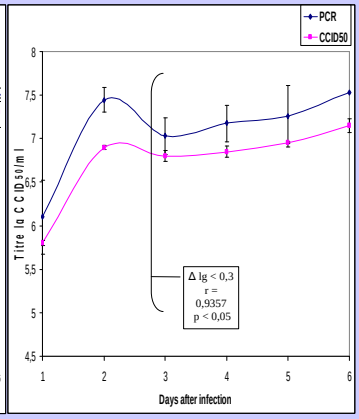

Fig.3. Growth curve for mumps virus as measured by qPCR-RT and $CCID_{50}$.

Fig.1, 2,3. Vero cells were infected with MOI 0,1 infectious units per cell.

The novel methods to determine the potency of MMR viruses in virus-containing bulks using qPCR-RT have been developed for each virus. It was found that the difference between viral titer measured by qPCR-RT and corresponding infectious viral titer measured by $CCID_{50}$ assay in paired samples did not exceed 0,3 $lgCCID_{50}$/ml (the WHO allowed value) for a period from 2 to 7 days after inoculation and the Pearson coefficient between results obtained by two assays was close to 1, indicating a significant correlation.

For the reference samples of vaccines, the discrepancy between the titers determined by qPCR-RT and $CCID_{50}$ assays was within 0,2 $lgCCID_{50}$/ml, which is less than the WHO limit.

Further, using quantitative real-time Multiplex PCR assay format it has been shown for laboratory variant of trivalent MMR vaccine (containing the L-16 measles, L-3 mumps and Wistar RA 27/3 rubella strains) and commercial batches of Measles-Mumps divalent and MMR trivalent (Priorix) vaccines that this approach can be used for quantitative analysis of MMR viruses and replication kinetics in infected cells.

It has to be noted that the significant results could only be obtained by the developed methods following the certain conditions such as:

- carrying out the potency estimation for virus bulks obtained between 2 and 7 days after inoculation;
- using laboratory references under the same conditions as virus samples;
- multiplicities of infection (MOI) of cell culture are not exceeding 0,1 infectious units per cell;
- storage of viral samples under certain conditions in order to exclude infectivity loss.

Conclusion

The new assay was shown to have high efficiency, high sensitivity and high reproducibility. This assay is virus-specific and serological neutralization can be omitted, besides it was faster and less laborious compared to the classical assay. The novel qPCR-RT method was demonstrated to be concordant with the classical assay. While the traditional methods would still be necessary to determine viral potency (in terms of infectious virus), the qPCR-RT allows for rapid determination of optimal harvest time and bulk virus titers.