A new qPCR-NGS Reflex ABL Kit to detect and identify Adenoviruses Types and Subtypes in Clinical Samples

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Introduction

Adenoviruses are one of the most common infectious pathogens detected in humans with confounding respiratory, conjunctival and diarrheal symptoms. Recently, a new severe and acute hepatitis in children was correlated to Adenovirus F-41 a virus normally linked to diarrheal symptoms. Accordingly, European and American CDCs declared a sanitary alert to survey uncommon hepatitis in children. Anticipating upcoming more thorough investigations about the possible involvement of Adenoviruses in various diseases, ABL diagnostics developed an “all-in-one” kit which detects all Adenoviruses and identify their types and subtypes.

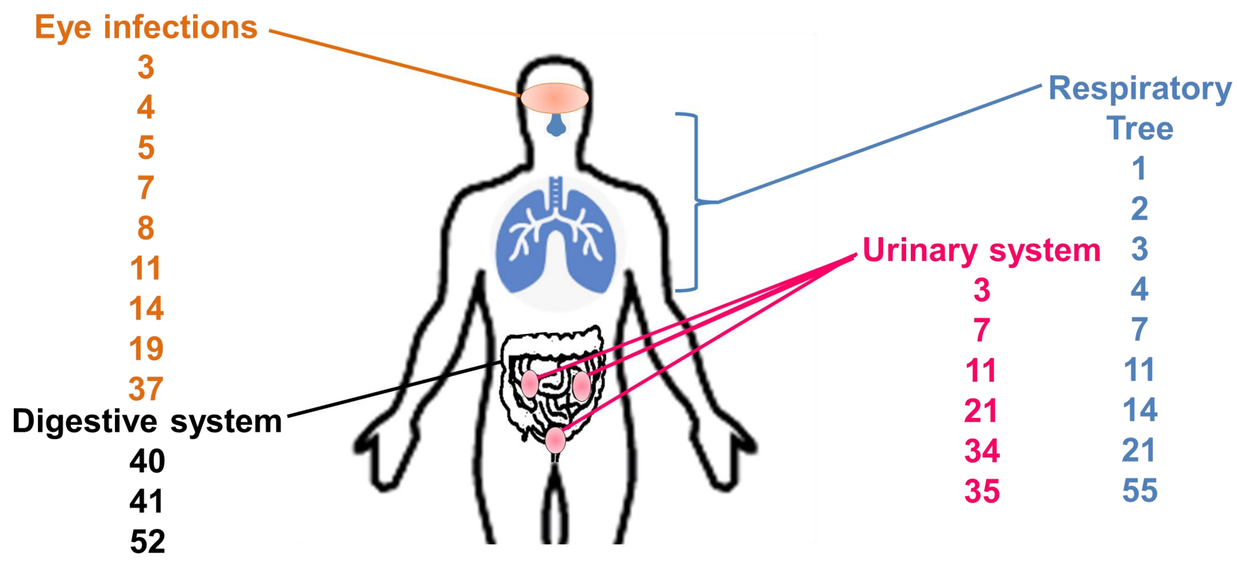

Figure 1: The tissues where Adenoviruses Subtypes induce symptomes. Adapted from presentation of Laetitia Ninove – IHU Infection Marseille, France

Methods

Supernatants of Adenoviruses 1,5,7,24,40,41 cultures and clinical sample / stool containing Adenoviruses were used to determine the capacity of Ultragene® Adenovirus qPCR assay of ABL to detect a wide diversity of Adenovirus, thermocycler CFX96 of Biorad company was used for amplification and fluorescence quantification. Amplicons of the qPCR were then sequenced using iSeq100 with the libraries were generated using the DeepChek® NGS Library preparation V2.

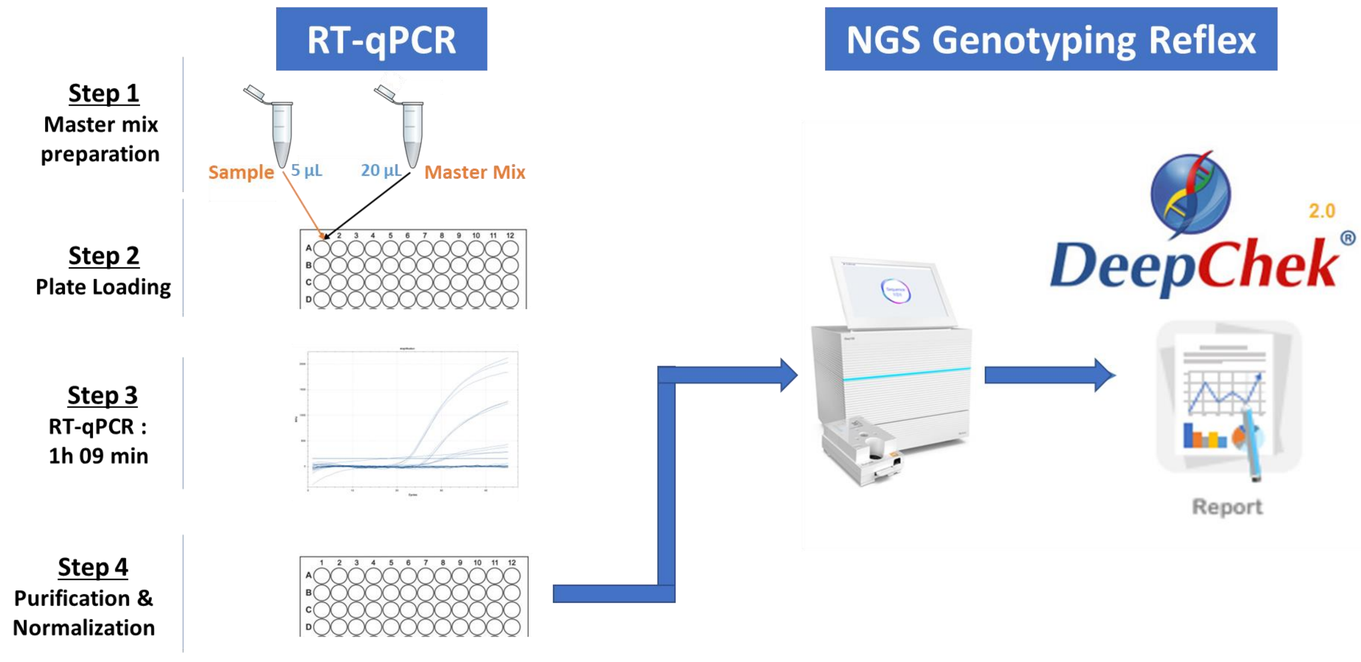

Figure 2: Diagnosis workflow

Results

- Ultragene® adenoviruses qPCR kit of ABL detected all adenoviruses tested (1, 5, 7, 24, 40, 41) with a limit of detection as low as $1.5x10^3$ $TCID_{50}$/mL for Adenovirus 40 and 5.6 $TCID_{50}$/mL for Adenovirus 41.
- The following NGS-Reflex generated sequences of the amplicon providing the identification of all types tested (B, C, D and F) and clearly identified Adenovirus subtypes 7, 40, 41.

Conclusions

- Adenovirus qPCR-NGS reflex kit could contribute to the worldwide effort to improve emerging diseases diagnosis where Adenoviruses are involved including this new hepatitis of unclear etiology correlated to Adenovirus 41.
- This workflow is compatible with diagnostic lab routine analysis.

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