

P0020 Development and evaluation of multiple real-time RT-PCR assays for simultaneous detection of enterovirus 71, coxsackievirus A16, coxsackievirus A6, coxsackievirus A10 and other enteroviruses associated with hand, foot and mouth diseaseWu Yidong*¹, Wei Yi², Shiyong Zhao²¹ Clinical laboratory, Hangzhou Children' Hospital, Hangzhou, ² Department of Infectious Disease, Hangzhou Children' Hospital, Hangzhou**Background:** EV71 and CA16 are the most common causes of hand, foot and mouth disease. CV-A6 and CV-A10 and other enteroviruses are increasing as causes year by year.**Materials/methods:** 930 throat swabs or stool specimens of children clinically diagnosed with hand, foot and mouth disease were collected randomly in Hangzhou Children's Hospital in 2017. The universal enterovirus (EV), enterovirus 71 (EV71), coxsackievirus A group 16 (CA16), coxsackievirus A group 6 (CA6) and coxsackievirus A group 10 (CA10) were simultaneously detected by real-time quantitative RT-PCR.**Results:** The total positive rate of 930 children with HFMD was 74.52% (693/930), of which EV71 accounted for 24.4% (169/693), CA16 for 4.3% (30/693), CA6 for 41.1% (285/693), CA10 for 6.1% (42/693) and other enteroviruses for 24.1% (167/693). The new 5-plex quantitative RT-PCR definite typing rate of HFMD enteroviruses was 75.9% (526/693), which was significantly higher than that of traditional triple quantitative RT-PCR typing rate (28.7% (199/693) ($\chi^2=308.558$, $P=0.000$). Compared with the traditional triple quantitative RT-PCR typing, the new 5-plex quantitative RT-PCR typing of enteroviruses increased the typing rate by 54.0%, 42.3% and 32.3% in the group of less than one year old, the group of 1-3 years old and the group of more than three years old, respectively. There was significant difference between the separation rate of the new 5-plex test and that of only EV71 typing in severe hand foot mouth disease ($\chi^2=5.368$, $P=0.021$). Multivariate regression analysis the relationship between severity and typing showed that the adjusted OR value of EV71 was 48.87 (95% CI: 26.12-91.43, $P = 0.000$), and the OR values of CA16, CA6 and CA10 were 3.148 (95% CI: 0.741-13.376, $P = 0.120$), 0.787 (95% CI: 0.263-2.358, $P = 0.669$) and 0.472 (95% CI: 0.056-3.984, $P = 0.490$) respectively.**Conclusions:** The etiology of hand, foot and mouth disease has gradually changed from the traditional common types of EV71 and CA16 to the antagonistic epidemics of EV71, CA6, CA16 and CA10. It is suggested that the new 5-plex quantitative RT-PCR assays for simultaneous detection of EV71, CA16, CA6, CA10 and other enteroviruses should be routinely practised in diagnosis of hand, foot and mouth disease.