

## 两种浓缩方法在水样脊髓灰质病毒检测中的应用

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## Application of Two Methods for Concentrating Viruses and Detection of Polioviruses in Water Samples

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## 摘要

水样中病毒颗粒的有效浓缩和富集是病毒检测的首要任务.分别应用氯化钠-氯化铝沉淀法和滤膜吸附法,对模拟水样和实际水样的脊髓灰质病毒进行浓缩分离.经过核酸提取后,通过RT-nest-PCR分子生物学技术扩增特异性核酸片段检测病毒,结果发现,两种浓缩方法对水样中的脊髓灰质病毒都能有效地回收富集及检测.通过计算,分别检测到污水处理厂进水中病毒存在所用水样的有效体积,氯化钠-氯化铝沉淀法为3.5 mL,滤膜吸附法为2.1 mL.应用氯化钠-氯化铝沉淀法对另外3个污水处理厂水样的病毒检测发现,进水和出水口均有脊髓灰质病毒的存在.另外,制备了包含脊髓灰质病毒特异性核酸片段的阳性质粒标准品,为开展水体环境中有害病毒的检测工作奠定研究基础.

关键词: 氯化钠-氯化铝沉淀法; 滤膜吸附法; 肠道病毒; 逆转录PCR; 巢式PCR

## Abstract:

Effective enrichment and recovery of virus particles from water is the primary task in virus detection in water samples. The present study used sodium chloride-aluminum chloride precipitation method and cation-coated filter method to concentrate polioviruses from a simulated virus contaminated water sample and a real untreated water sample collected from a sewage treatment plant in Shanghai. RNA was extracted from concentrated viruses and then reverse transcription-PCR (RT-PCR) and nest PCR were performed. The results revealed that polioviruses could be detected from the real water sample by means of two concentration methods, indicating that viruses had been effectively recovered from water by those two methods. The presence of polioviruses was detected from virtual volume of 3.5 mL untreated water sample using sodium chloride-aluminium chloride precipitation method and from virtual volume of 2.1 mL untreated water sample using cation-coated filter method. We also found the presence of polioviruses in the influent and effluent water samples collected from another three sewage treatment plants using sodium chloride-aluminium chloride precipitation method and RT-nest-PCR detection. In addition, a recombinant plasmid containing poliovirus-specific fragment was constructed and prepared as a positive experimental control for the further poliovirus detection. Our study provided preliminary data for applications of the two different methods for virus concentration in water samples and observed potential enterovirus pollution in aquatic environment in Shanghai.

Keywords: NaCl-AlCl<sub>3</sub> precipitation method; cation-coated filter method; enterovirus; reverse transcription-PCR (RT-PCR); nest PCR

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