Purification and Analysis of dsRNA

Highly purified rotaviral dsRNA can be prepared directly from fecal samples using the guanidinium–phenol technique followed by binding to silica particles in the presence of chaotropic agents. The guanidine thiocyanate–phenol reagent is conveniently supplied as RNAzol reagent by Cinna/Biotecx Laboratories. However, after the addition of chloroform and removal of the aqueous phase containing the RNA, the manufacturers recommend precipitation with isopropanol to recover the RNA. We have modified the procedure to make use of the observation by Boom *et al.* (6) that rotaviral dsRNA will bind to silica particles in the presence of high concentrations of sodium iodide. This procedure permits extensive washing of the RNA in chaotropic reagents to remove potential inhibitors of the RNA ligase reaction. It is critical to extract the dsRNA exactly as described, because RNA extracted by the more usual technique of phenol extraction and ethanol precipitation does not accept primer 1 in the RNA ligation reaction. It is also advisable to check the quality and quantity of dsRNA by polyacrylamide gel electrophoresis (PAGE) before proceeding with the ligation reaction.

Extraction of dsRNA

This method works best on clinical specimens that are preferably less than 0.5 ml in volume, so that all the manipulations can be performed in a 1.5-ml microfuge tube. It is also preferable that the samples contain at least 10^9 rotavirus particles to allow visualization of the extracted dsRNA by silver staining (7). In addition, pretreatment of the fecal sample with RNase A or RNase T1 to remove contaminating rRNA or tRNA prior to guanidinium–phenol extraction gives a much cleaner genomic RNA profile as judged by sodium dodecyl sulfate (SDS)–PAGE and silver staining.

Reagents and Solutions

RNAzol B
Geneclean (Bio 101)
RNase T1 [GIBCO—Bethesda Research Laboratories (BRL), Gaithersburg, MD]
New Wash (NaCl–ethanol–water; provided in the Geneclean kit)

Method

1. Add 1 μl (10,000 U) of RNase T1 to a liquid fecal suspension (~50%). Bring the sample volume up to 0.5 ml with sterile water.
2. Incubate at 55° C for 15 min.
3. Add 0.5 ml of RNAzol B and mix thoroughly.