Serum Albumin Treatment To Eliminate False-Positive Results with Laboratory Rodent Specimens Tested by Rotazyme Enzyme-Linked Immunosorbent Assay

MARIA NOEL JURE, STEPHEN S. MORSE, AND DENNIS M. STARK*

Laboratory Animal Research Center, The Rockefeller University, New York, New York 10021-6399

Received 17 July 1987/Accepted 11 August 1987

A high proportion of positive results was found in fecal specimens from mice and rats tested by Rotazyme II enzyme-linked immunosorbent assay, but the presence of virus could not be confirmed. Positive specimens and nonautoclaved rodent diet contained a substance that apparently attached nonspecifically to the antibody-coated beads used in the test and reacted directly with the substrate. Pretreatment of the beads with 0.1% bovine serum albumin eliminated this nonspecific activity.

Rotaviruses are among the most important causes of neonatal gastroenteritis in many animal species and in humans (2, 5). Enzyme-linked immunosorbent assay (ELISA), with its advantages of specificity and simplicity, has become the predominant method for rotavirus screening (11). Rotazyme II (Abbott Laboratories, North Chicago, Ill.), which uses polystyrene beads coated with anti-rotavirus antibody to bind rotavirus antigens present in the test samples, is the assay most widely used to identify rotaviruses in humans and has also been used for the detection of rotaviruses in several animal species, including rodents (7).

During the course of testing rotavirus prevalence in laboratory animals received at The Rockefeller University Laboratory Animal Research Center, we found large numbers of positive samples in mouse and rat specimens tested by Rotazyme. These false-positive reactions appeared to be caused by a substance present in food and stool samples which could attach nonspecifically to the antibody-coated beads and react directly with the substrate. We report here that serum albumin pretreatment of the beads eliminated these false-positive results.

For rotavirus testing by Rotazyme II, 10% suspensions of mouse or rat fecal specimens or of food samples were prepared by trituration in Rotazyme II sample diluent (phosphate-buffered saline, pH 7.2; Abbott Laboratories) and were clarified by centrifugation at $1,000 \times g$ for 10 min, and the supernatants were used. To provide known positive samples, a rotavirus pool was made from intestines of mice infected with epizootic diarrhea of infant mice (EDIM) (kindly provided by Abigail Smith, Yale University, New Haven, Conn.) using aseptic techniques. The intestines were ground in hand-held Ten Broeck tissue grinders with Rotazyme sample diluent added to produce a 10% (wt/vol) suspension. The suspension was centrifuged at $1,000 \times g$ for 10 min to remove the debris. The supernatant was positive, with a Rotazyme titer of 0.660. Neonatal mice (5 to 7 days old) were inoculated perorally with 0.05 ml of this material with a gavage needle. Known virus-positive fecal samples were prepared by collecting fecal samples directly from the rectum at 6 days postinoculation. For virus pools, pooled intestines were removed and stored at -70°C . Control pups were inoculated per os with 0.05 ml of sample diluent. The Rotazyme II ELISA (Abbott Laboratories) was performed

according to the instructions of the manufacturer with the Abbott reagents, and samples were read in the Quantum II spectrophotometer (Abbott Laboratories) at 492 nm (A_{492}). For bovine serum albumin (BSA) pretreatment, beads were incubated in 200 μl of 0.1% BSA (catalog no. A 7030; Sigma Chemical Co., St. Louis, Mo.) per bead, in the Rotazyme ELISA plate, for 1 h at 37°C . Beads were then washed three times with distilled water. Each specimen was tested in duplicate with pretreated and untreated beads. The remainder of the assay was as described above.

In the process of testing randomly collected stool specimens from laboratory animals received at this facility, we found that one-third of 62 mouse samples and 82% of 72 rat samples tested positive by Rotazyme. However, no rotavirus could be detected in these samples by standard confirmatory tests (1, 3, 6, 8), including a blocking-type ELISA with anti-EDIM serum, electron microscopy, or virus isolation in primary African green monkey kidney or MA104 cells.

Rotazyme conversion was correlated with the animals being fed nonautoclaved food. Of the mice that received nonautoclaved food, 41.2% were positive with a mean A_{492} of 0.153. All mice receiving only autoclaved chow tested negative at all times. When extracts made from different batches of food were tested, all unheated food samples were positive (mean A_{492} , 0.82). After autoclaving (130°C , 30 min), samples became negative, as did positive rat or mouse stool samples. Positive food and stool suspensions also gave positive reactions when tested by Rotazyme in the absence of the conjugate, suggesting that a substance in these samples reacted with the assay substrate after attaching nonspecifically to the Rotazyme assay beads.

Pretreatment of the beads with 0.1% BSA eliminated the nonspecific activity of food and stool samples (Table 1). BSA-treated beads and untreated beads gave similar results when positive-control antigen preparations or stool samples from experimentally infected litters were tested. All tested positive; treated and untreated beads gave similar results (Table 1). BSA pretreatment of beads appeared more effective than incorporating BSA in the sample diluent without treating the beads (Table 1).

To verify that BSA treatment could distinguish the presence of viral antigen from the false-positive material, a known amount of purified control antigen was added to a false-positive stool sample that showed nonspecific activity

* Corresponding author.

TABLE 1. Effect of BSA treatment on Rotazyme assay

Sample ^a	Mean A ₄₉₂		
	Standard Rotazyme	Rotazyme with BSA in diluent only ^b	Rotazyme with BSA-treated beads ^c
Mouse stool	0.193	0.155	0.089
Rat stool	0.238	0.110 ^d	0.067
Food extract	0.642	0.256	0.090
Positive-control antigen ^e	0.976	ND ^f	0.920
Stool from infected litters	0.837	ND	0.765
Stool from control litters	0.036	ND	0.032

^a Minimum of 10 samples in each group.^b Standard Rotazyme II assay and untreated beads, with BSA (0.1%) in the diluent.^c Rotazyme assay beads were pretreated with BSA (0.1%) as described in the text.^d Control for these samples was 0.148.^e Rotazyme rotavirus positive-control antigen (Abbott Laboratories).^f ND, Not determined.

when tested in the absence of the conjugate. This sample, plus a known amount of the positive-control sample, was run in duplicate with BSA-pretreated beads and untreated beads (standard Rotazyme). The Rotazyme titer of the original sample was 0.104. An amount of positive-control sample with an A₄₉₂ of 0.274 was added. The Rotazyme titers of the mixed sample were 0.262 when the sample was tested with BSA-pretreated beads and 0.373 in the standard Rotazyme.

Rotazyme is a commercially available rotavirus ELISA that is widely used in human medicine and that also has been used in laboratory animals (7, 9). In human studies, Rotazyme was shown to be a reliable and sensitive immunologic test, as confirmed by electron microscopy (1, 8). However, occasional false-positive reactions have been found in human stool specimens (4, 12). False-positive reactions can be a significant problem in general in ELISA, particularly for certain types of samples (10, 11). Nonspecific reactions were observed in mouse and rat stool samples tested in this laboratory by Rotazyme. These false-positive reactions in mouse and rat stool samples appeared to be due to a component derived from the nonautoclaved food. Both food extracts and positive stool samples could produce color when added directly to the *o*-phenylenediamine substrate.

BSA pretreatment of Rotazyme-coated beads successfully prevented the false-positive reactions and did not affect the specific activity of known rotavirus-positive samples. BSA is widely used in enzyme immunoassays to saturate unbound sites and to reduce nonspecific binding (10). BSA was less

effective when used in the sample diluent without pretreating beads. The data presented here indicate that BSA pretreatment of Rotazyme beads eliminates the nonspecific reactions, making this assay suitable for laboratory animal samples. It is also likely to increase the specificity and reliability of the test with other types of questionable samples.

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