Radioimmunoassay of Measles Virus Nucleocapsid Antigen

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SUMMARY

A double antibody competitive binding radioimmunoassay (RIA) was developed as a tool for investigating the involvement of measles virus in persistent virus infections. The assay employs 125I-labelled measles virus nucleocapsids as the labelled antigen. Nucleocapsids were purified from cytoplasmic extracts of virus-infected Vero cells, treated with trypsin to prevent clumping and iodinated to a specific activity of about 15 μCi/μg. Electrophoretic analysis of iodinated trypsin-treated nucleocapsids revealed only labelled polypeptide with a relative mol. wt. (M,) 40000, indicating that trypsin had cleaved the 60000 mol. wt. native polypeptide to a 40000 mol. wt. subunit. The assay could detect as little as 0.1 ng of nucleocapsid protein. Either native (M, 60000) or cleaved (M, 40000) nucleocapsid polypeptide was detected in the assay. As much as 100 μg of protein from uninfected Vero cells did not react in the RIA. Another measure of specificity was the fact that 400 ng of purified nucleocapsid protein from simian virus 5, Newcastle disease virus or canine distemper virus did not react in the assay. In the RIA, nucleocapsid antigen of virus isolated from subacute sclerosing panencephalitis (SSPE) was indistinguishable from that of Edmonston strain measles virus, indicating antigenic homology between the 40000 mol. wt. nucleocapsid polypeptide of the SSPE virus and that of measles virus.

INTRODUCTION

Highly sensitive and specific assays for measles virus products are essential in order to investigate adequately the involvement of measles virus in subacute sclerosing panencephalitis (Connolly et al. 1967; Horta-Barbosa et al. 1969; Payne et al. 1969) and the possible role of measles virus in multiple sclerosis (Norrby et al. 1974; Haire, 1977). Radioimmunoassay (RIA) is a highly sensitive technique which has been used to detect, measure and characterize a variety of antigens. In the present study, a competitive binding RIA for the major measles virus nucleocapsid antigen was developed and evaluated. Nucleocapsid antigen was the target of this study because nucleocapsids are commonly present in persistent measles virus infections where infectious virus is absent or difficult to detect (Payne et al. 1969; Knight et al. 1973–74).

We chose to use nucleocapsids rather than soluble nucleocapsid polypeptide as labelled antigen in the RIA because of the relative ease with which nucleocapsids can be purified. However, we were unsuccessful in isolating nucleocapsids containing only native, 60000 mol. wt. polypeptide. The use of protease-inhibitors to preserve the native polypeptide during purification yielded nucleocapsids that aggregated and contained at least three polypeptides by gel electrophoresis. However, trypsin-treatment of cytoplasmic extracts of...
infected cells made isolation of nucleocapsids that did not aggregate and that contained only a 40,000 mol. wt. subunit of the major nucleocapsid polypeptide possible. Although we were not able to label these purified trypsin-treated nucleocapsids with $^{125}$I using either the chloramine-T (Hunter & Greenwood, 1962) or the lactoperoxidase (Marchalonis, 1969) methods, we were successful in labelling them by using the Bolton–Hunter reagent, an iodinated acylating intermediate (Bolton & Hunter, 1973). Such $^{125}$I-labelled trypsin-treated nucleocapsids proved to be appropriate as the labelled antigen in the competitive binding assay.

**METHODS**

**Cells.** Vero cells were propagated in Eagle’s minimal essential medium (MEM) containing 10% foetal calf serum (FCS) and maintained in MEM and 2% FCS. All media contained 100 units of penicillin and 100 μg of streptomycin per ml. Cells were grown in plastic tissue culture flasks (75 cm$^2$) or glass roller bottles (0.5 gallon).

**Viruses.** Non-attenuated Edmonston strain measles virus was originally obtained from Dr H. M. Myers, Jun. (Bethesda, Md., U.S.A.) and passaged six times in BSC-1 cells and four times in Vero cells. SSPE virus was isolated from a patient with subacute sclerosing panencephalitis (SSPE) by co-cultivating brain cells with human embryo kidney cells and was designated MUN-HT (Payne & Baublis, 1973). The virus was passaged three times in human embryo kidney cells followed by six passages in BSC-1 cells and four passages in Vero cells. The W-3 strain of simian virus 5 (SV5) was obtained from Dr M. P. Kiley (Atlanta, Ga., U.S.A.) and propagated in Vero cells. Newcastle disease virus (NDV) supplied by Dr H. F. Maassab (Ann Arbor, Mich., U.S.A.) had been passaged three times in HeLa cells, once in chick kidney cells and once in the allantoic fluid of chicken eggs before passage in Vero cells. Canine distemper virus (CDV; Green’s distemperoid strain, chick tissue culture adapted) was obtained as vaccine from Fromm Lab, Inc. (Grafton, Wisc., U.S.A.) and passaged once in Vero cells.

**In culture labelling of nucleocapsids.** Measles or SSPE virus was diluted to give $5 \times 10^6$ p.f.u./ml and 0.5 ml (6 ml for roller bottles) was added to 11- to 14-day-old Vero cell cultures and incubated at 36 °C for a 3 h adsorption period. A 1:10 dilution of a Vero cell passaged pool of CDV, NDV or SV5 was used to infect Vero cells in the same manner. After the adsorption period, cultures were washed with Hanks’ balanced salt solution and 10 ml (50 ml for roller bottles) of maintenance medium added. Cultures infected with measles virus were incubated further at 36 °C for 24 h and cultures infected with SSPE virus, CDV, NDV or SV5 were incubated for 48 h. At the end of that time the maintenance medium was replaced with 8 ml (50 ml for roller bottles) of MEM containing 7% of the normal concentration of amino acids and 2% dialysed FCS. $^{3}$H-amino acids (reconstituted protein hydrolysate, Schwarz/Mann, Orangeburg, New York) were then added to the medium to a concentration of 0.05 mCi/ml. Cultures were allowed to incubate at 36 °C for an additional 16 h (40 h for CDV-infected cultures).

**Purification of nucleocapsids.** To prepare measles nucleocapsids containing the 40,000 mol. wt. subunit of the nucleocapsid polypeptide, infected cells were removed from the surface of culture bottles with glass beads and washed in cold tris-buffered saline, pH 7.4 (TBS: 0.01 M-tris, 0.14 M-NaCl). The cells were sedimented at 1,500 g for 10 min and resuspended in hypotonic buffer (HB: 0.01 M-tris, 0.01 M-NaCl, pH 7.4). Cells were allowed to swell for 10 min and then disrupted with 10 strokes in a Dounce homogenizer. Nuclei were removed by centrifugation at 1,500 g for 10 min. The supernatant fluid of this cytoplasmic extract was treated with 0.5 mg/ml crystalline pancreatic trypsin (Sigma, St. Louis, Mo., U.S.A.) for 10 min at 25 °C followed by 0.5 mg/ml soybean trypsin inhibitor
RIA of measles virus nucleocapsid antigen

(Sigma). Deoxycholate (Sigma) and BRIJ-58 (Atlas Biochemical Industries, Wilmington, Delaware) were added to the fluid to give a final concentration of 1-0% of each detergent. After EDTA was added to the fluid to give a concentration of 0.06 M, the cytoplasmic extract was layered on a 15 to 40% (w/v) sucrose/HB-0.06 M-EDTA, pH 7.4 gradient. The gradient was centrifuged in the SW27 rotor at 95,000 g for 4 h in a Beckman L3-50 ultracentrifuge. Fractions of 1.5 ml were collected from the bottom of the gradient and acid-precipitable radioactivity determined as described previously (Kiley et al. 1974). Nucleocapsids were harvested from the 200S region of the gradient. The trypsin-treated nucleocapsids of CDV, NDV and SV5 were purified in the same manner. Measles virus nucleocapsids containing the 60,000 mol. wt. native polypeptide were prepared in the same manner except that infected cells were washed in TBS containing 10^{-4} M-α-toluene-sulphonyl fluoride (α-TSF, Eastman Kodak, Rochester, New York) and swollen in HB containing 10^{-4} M-α-TSF. Also, EDTA was added to the cytoplasmic extract and the sucrose gradient to give a final concentration of 0.02 M.

Trypsin-treated measles virus nucleocapsids, which were to be labelled with ^{125}I, were further purified by equilibrium centrifugation. Continuous sucrose gradients, 20 to 65% (w/w) made in NTE (0.1 M-NaCl, 0.01 M-tris, 0.001 M-EDTA, pH 7.4) were centrifuged for 5.5 h at 78,000 g in an SW27-1 rotor. Fractions of 0.75 ml were collected and acid-insoluble radioactivity determined. Nucleocapsids banding at a density of 1.3 g/ml were harvested and dialysed overnight in a 0.1 M-borate buffer, pH 8.5. The volume of the dialysate was reduced with polyethylene glycol so that concentration of nucleocapsids in borate buffer was about 0.1 mg/ml.

In vitro labelling of nucleocapsid antigen with ^{125}I. The trypsin-treated nucleocapsids (7.5 μg in 75 μl 0.1 M-borate buffer, pH 8.5) were iodinated to a sp. act. of 15 μCl/μg by the method of Bolton & Hunter (1973). The products of the reaction mixture were separated by column chromatography on a Bio-Gel P-30 column equilibrated with 0.05 M-phosphate buffer, pH 7.4 containing 0.2% pigskin gel (Eastman Kodak, Rochester, New York). Fractions from the first peak of radioactivity eluting from the column were combined and layered on a 15 to 40%, w/v, sucrose/HB-0.06 M-EDTA gradient, pH 7.4, and centrifuged at 90,000 g for 3 h at 4 °C in an SW27 rotor. Fractions (1.5 ml) were collected and the amount of radioactivity in each fraction determined. Material from the 200S region of this gradient was diluted in phosphate-buffered saline (PBS), pH 7.4 containing 0.1% bovine serum albumin (BSA) so that 100 μl contained 3500 ct/min. This material was used as labelled antigen in the RIA.

Polyacrylamide gel electrophoresis (PAGE) was performed according to the method of Maizel (1971) in a discontinuous tris buffer system. Gels consisted of a 2.5% acrylamide stacking gel layered over a 7.5% separating gel. Samples (100 μl) were dissociated by boiling for 2 min in 0.06 M-tris (pH 7.4) containing 1% SDS, 1% mercaptoethanol and 8 M-urea. These samples were electrophoresed for 4 h at a constant current of 4 mA/gel, removed from the gel tubes and cut into 1 mm slices. For ^{125}I-labelled preparations, sequential pairs of gel slices were counted in a Packard Auto Gamma Spectrometer. For ^{3}H-labelled preparations, sequential pairs of gel slices were dissolved by incubation in 50 μl of H_{2}O_{2} at 60 °C for 16 h. Protosol (0.5 ml) and a toluene-based scintillation counting fluid were sequentially added to the dissolved gels and the amount of radioactivity determined.

Anti-nucleocapsid serum. A preparation of purified trypsin-treated measles nucleocapsids was emulsified with an equal amount of Freund’s complete adjuvant. This emulsion was injected into 3-month-old male New Zealand rabbits on days 1, 18, 42 and 209. The first three injections each contained approx. 200 μg of antigen and the fourth 100 μg of antigen. Each injection (2.0 ml) was divided equally between the hind footpads and 20 intradermal
sites on the rabbit's back. Sera used in the present study were derived from blood drawn on the 216th day after the first injection.

Rabbit antisera were titred by radioimmunoprecipitation. The sera were serially diluted in PBS-o.05 M-EDTA, pH 7.4, containing 0.25% normal rabbit serum. 125I-nucleocapsids (3500 ct/min) in 0.6 ml of PBS-o.1% BSA were mixed with 0.2 ml portions of diluted rabbit anti-nucleocapsid serum and incubated for 18 h. Control tubes received 0.2 ml of diluent instead of 0.2 ml of diluted anti-nucleocapsid serum. Sheep anti-rabbit-globulin (SAG), 0.2 ml diluted 1:5 in PBS-o.05 M-EDTA, pH 7.4, was added to precipitate the complex and incubated for 1 h at 25 °C and 3 h at 4 °C. (SAG was kindly supplied by Dr B. England, Ann Arbor, Mich. The optimal dilution of SAG was determined by assessing the ability of various dilutions of the SAG to precipitate rabbit anti-nucleocapsid serum complexed to 125I-nucleocapsids.) The precipitate was then diluted by adding 3 ml of PBS, pH 7.4, to each tube. Precipitates were sedimented by centrifugation at 1500 g for 30 min and radioactivity in the precipitates was measured. Counts precipitated in the control tubes (about 12% of those added) were subtracted from counts precipitated by the anti-nucleocapsid serum in calculating the percentage of antigen precipitated by the antiserum.

**Competitive binding assay.** Decreasing volumes of PBS-o.1% BSA (0.5 to 0.001 ml) were added to a series of tubes. This was followed by the addition of increasing volumes (0 to 0.5 ml) of a given concentration of test antigen diluted in PBS-o.1% BSA to give a total volume of 0.5 ml. Each tube then received 0.2 ml of a 1:64000 dilution of rabbit anti-nucleocapsid serum which had previously been determined to precipitate 40% of the added 125I-nucleocapsid antigen. After mixing, all tubes were allowed to incubate for 18 h at 4 °C. 125I-nucleocapsids (3500 ct/min) in 0.1 ml of PBS-o.1% BSA were added to each tube. After mixing, all tubes were incubated for an additional 18 h at 4 °C. Two-tenths ml of sheep anti-rabbit-globulin diluted 1:5 in PBS-o.05 M-EDTA, pH 7.4, was then added to each tube. After mixing, the tubes were incubated at 25 °C for 1 h and at 4 °C for 3 h. The precipitate was then diluted, sedimented and counted as described above.

**Cytoplasmic extract from uninfected cells.** Confluent monolayers of uninfected Vero cells were harvested with glass beads and washed with TBS. Cells were then swollen in HB and homogenized. The nuclei were pelleted by centrifugation at 1500 g for 10 min. The supernatant fluid was extracted with double its volume of ethyl ether. Deoxycholate and BRIJ-58 were added to the aqueous layer to give a concentration of 0.1% of each detergent. The protein concentration in this cytoplasmic extract was determined using the method of Lowry et al. (1951).

**RESULTS**

**In vitro labelling and characterization of nucleocapsids**

Trypsin-treated nucleocapsids were efficiently labelled with 125I by using the Bolton-Hunter reagent (Bolton & Hunter, 1973). Nucleocapsids iodinated in this manner and separated from the reaction mixture by column chromatography on Bio-Gel P-30 were examined by velocity sedimentation on a 15 to 40% (w/v) sucrose gradient and found to sediment in the 200S region. PAGE was performed on material from the 200S region of such a gradient and only iodinated polypeptide with a Mr of 40000 was found (Fig. 1).

**Immunoprecipitation of 125I-nucleocapsids**

The immunoreactivity of 125I-labelled trypsin-treated nucleocapsids was examined by double-antibody precipitation. The ability of various dilutions of rabbit anti-nucleocapsid serum to precipitate 125I-nucleocapsids is illustrated in Fig. 2. At high concentrations of rabbit anti-nucleocapsid serum, 125I-nucleocapsids were over 80% precipitable. As seen in
RIA of measles virus nucleocapsid antigen

Fig. 1. PAGE of ¹²⁵I-labelled trypsin-treated measles virus nucleocapsids. After separation from other products of the labelling reaction by column chromatography on a Bio-Gel P-30 column, ¹²⁵I-labelled nucleocapsids were further purified by centrifugation on a 15 to 40% (w/v) sucrose/HB-0.06 M-EDTA, pH 7.4, gradient for 3 h at 4 °C. Material banding in the 200S region was treated with 0.1% SDS and 0.1% ME and electrophoresed for 4 h at 4 mA/gel on a 7.5% polyacrylamide cylindrical gel. Gels were sliced and the amount of radioactivity determined. Vesicular stomatitis virus (VSV) ¹⁴C-amino acid labelled proteins (indicated by arrows) were run in parallel as markers. Mol. wt. were assigned to the VSV proteins as noted by Knipe et al. (1975).

Fig. 2. Precipitation of ¹²⁵I-labelled measles nucleocapsid antigen by various dilutions of rabbit anti-nucleocapsid serum.

Fig. 2, the rabbit anti-nucleocapsid serum, even at a dilution of 1:80000, precipitated as much as 80% of the ¹²⁵I-nucleocapsids. Pre-immune rabbit serum diluted 1:400 precipitated less than 12% of the ¹²⁵I-nucleocapsids. Having established that the ¹²⁵I-nucleocapsids were specifically precipitated by the rabbit anti-nucleocapsid serum, these reagents were used in subsequent competitive binding studies.

Competitive binding of nucleocapsid antigen (the radioimmunoassay)

Competitive binding curves were developed in order to assess the sensitivity of the procedure for detecting antigen. Various amounts of purified trypsin-treated measles nucleocapsid antigen were incubated with samples of dilute rabbit anti-nucleocapsid serum. A 1:640000 dilution of antiserum which had been previously found to precipitate 40% of the ¹²⁵I-nucleocapsids was used. After incubation of the mixtures for 18 h, ¹²⁵I-nucleocapsids were then added to detect unbound antibody. Fig. 3 is a log-logit plot of a competitive binding curve. Precipitation of ¹²⁵I-nucleocapsids was 12% inhibited by 0.1 ng of nucleocapsid protein. At 20 ng of nucleocapsid protein there was complete inhibition of precipitation of the labelled antigen. These results indicate that the sensitivity of the assay, about 0.1 ng of protein, compares favourably with that of other competitive binding assays.

Specificity of the RIA

It was found that 100 µg of protein extracted from uninfected Vero cells did not react in the RIA, nor did that amount interfere with detection of trypsin-treated measles nucleo-
Fig. 3. Competitive binding of trypsin-treated measles virus nucleocapsid antigen (log-logit plot). Increasing amounts of measles nucleocapsids were mixed with a standardized amount of rabbit anti-nucleocapsid serum. This mixture was allowed to incubate at 4 °C for 18 h. 125I-labelled measles nucleocapsids were then added to determine the amount of unbound antibody. Results are expressed as percent maximum precipitation of 125I-nucleocapsids.

Fig. 4. Velocity sedimentation of 3H-amino acid-labelled nucleocapsids from cytoplasmic extracts of measles infected cells isolated in the presence of trypsin. Cytoplasmic extract was layered on to a 38 ml 15 to 40 % (w/v) sucrose gradient made up in HB-o.06 M-EDTA, pH 7.4. The gradient was centrifuged at 90000 g for 4 °C in an SW27 rotor. Acid-precipitable radioactivity (O--O) and nucleocapsid antigen (■—■) were determined by TCA precipitation and RIA respectively. Capsid antigen. Larger amounts of uninfected cell protein were not tested. As little as 0.1 ng of nucleocapsid antigen was detectable in a 10^4-fold excess of tissue protein.

To examine further the specificity of the RIA, the ability of various forms of the measles nucleocapsid antigen to react in the assay was tested. As shown in Fig. 4, at 40 h p.i. a trypsin-treated preparation of cytoplasmic extract of cells infected with measles virus and labelled in vivo with 3H-amino acids yielded a major peak of nucleocapsid antigen and a coincident peak of acid-precipitable 3H in the 200S region of a sucrose gradient. In addition, there was a small peak of nucleocapsid antigen detected in the 100S region of a sucrose gradient. This peak was not well defined by acid-precipitable 3H. Short nucleocapsids sedimenting at about 100S have been described previously (Kiley et al. 1974). Compared to the 200S and 100S peaks, a relatively small amount of nucleocapsid antigen was detected in the upper region of the gradient where there was a large amount of acid precipitable 3H. Fig. 5 shows that trypsin-treated nucleocapsid antigen from
RIA of measles virus nucleocapsid antigen

Fig. 5. Competitive binding of trypsin-treated measles virus nucleocapsid antigen (40000 mol. wt.) from < 4S (©--©) and 200S (■—■) fractions (log-logit plot). Increasing amounts of competing antigen were assayed as described for Fig. 3.

Fig. 6. Competitive binding of trypsin-treated nucleocapsids from NDV (○ -- ○), SV5 (△—△), CDV (× — ×), SSPE (● — ●) and measles (○ — ○) virus-infected cells. Increasing amounts of competing antigens were assayed as described for Fig. 3.

either the 200S or the < 4S (soluble) region of the gradient reacted in the RIA such that the slopes of the competition curves were indistinguishable. Native 60000 mol. wt. nucleocapsid antigen from either the 200S or < 4S region of a sucrose gradient reacted in the RIA such that the slope of the competition curve was the same as that of the curves produced by trypsin-treated nucleocapsid antigen.

Trypsin-treated nucleocapsid antigens from various paramyxoviruses were tested in the assay. As can be seen in Fig. 6, partially purified trypsin-treated nucleocapsids from SV5 and NDV did not react in the RIA. Even trypsin-treated nucleocapsids from CDV, a virus which is antigenically related to measles, did not react in the RIA indicating a high degree of specificity.

Comparison of nucleocapsid antigen of conventional measles virus and SSPE virus

SSPE viruses, isolated from patients with subacute sclerosing panencephalitis, are serologically very similar to measles virus (Horta-Barbosa et al. 1970; Payne & Baublis, 1973). Fig. 6 shows that trypsin-treated nucleocapsids from cells infected with SSPE virus and nucleocapsids from cells infected with conventional measles virus reacted in the RIA such that the slopes of the competition curves were identical. A log–logit plot of the same data confirmed the parallelism of the competition curves indicating antigenic homology.

To compare further the nucleocapsid antigens of these two types of viruses, competition curves were developed in an RIA which substituted antibodies from an SSPE patient for rabbit anti-nucleocapsid serum. This substitution was possible because sheep anti-rabbit globulin was found to cross-react with human gamma globulin in the assay (L. S. Amesse,
unpublished data). The competition curves of the two antigens again had the same slope. These results, like those obtained using rabbit anti-nucleocapsid serum in the assay, indicated that trypsin-treated nucleocapsids from SSPE and measles virus are antigenically very similar. In addition, the results suggested that antibodies from the SSPE patient, like antibodies from hyperimmune rabbits, had a high affinity for nucleocapsid antigen.

DISCUSSION

In evaluating the RIA, described in the present study, it was critical to establish the ability of various forms of the measles nucleocapsid antigen to react in the assay. Although the assay employed 200S nucleocapsids rather than 'soluble' polypeptide as labelled antigen, it was found to detect nucleocapsid antigen from the < 4S as well as the 200S regions of sucrose gradients of cytoplasmic extracts of cells infected with measles virus. This observation was true when the nucleocapsid polypeptide was present as either the native 60000 mol. wt. or cleaved 40000 mol. wt. form. Nucleocapsid polypeptide in the 200S region of the gradients is associated with virus RNA as nucleocapsids. Soluble nucleocapsid polypeptide would be expected to be present in the upper region, e.g. the < 4S region, of the gradients.

The high degree of specificity of the RIA was reflected in several observations. Large amounts of protein from uninfected cells did not react or interfere in the assay. Nucleocapsids of NDV and SV5, paramyxoviruses with no known antigenic relationship to measles virus, did not react. Most striking was the observation that nucleocapsids of CDV, which are known to cross-react antigenically with measles nucleocapsids, did not react in the RIA. Orvell & Norrby (1974) noted cross-reactivity between the nucleocapsid antigens of measles and CDV when tested by fluorescent antibody, immunodiffusion or complement fixation techniques. The rabbit anti-measles nucleocapsid serum used in the present study, when present in relatively high concentration precipitated trypsin-treated CDV nucleocapsids but, apparently because cross-reacting antibodies were diluted out in the RIA, CDV nucleocapsids did not react in the RIA. It is possible that trypsin-treatment of the nucleocapsids used in the RIA removed antigenic determinants which would cross-react in other techniques for antigenic comparison.

SSPE virus has been shown to be morphologically and antigenically similar to measles virus (Horta-Barbosa et al. 1970; Payne & Baublis, 1971). However, some antigenic differences between the two viruses were detected by neutralization assay (Payne & Baublis, 1973). Recently, Wechsler & Fields (1978) and Hall et al. (1978) reported that the M protein of SSPE virus differs from that of measles virus in size and antigenicity. In the present study, trypsin-treated nucleocapsids from cells infected with SSPE or with measles virus sedimented in the 200S region of sucrose gradients. The electrophoretic profile of material from the 200S region of gradients of either SSPE or measles virus-infected cells showed a single polypeptide of M, 40000. Nucleocapsids from cells infected with SSPE virus or measles virus reacted in the RIA such that the slopes of the competitive binding curves were indistinguishable. The fact that the slopes of the competitive binding curves were identical and that nucleocapsids from either virus completely inhibited binding of the labelled antigen by specific antibody indicates antigenic homology between the 40000 mol. wt. nucleocapsid polypeptide of SSPE virus and that of measles virus.

The RIA that makes use of 125I-labelled nucleocapsids containing the 40000 mol. wt. proteolytic subunit of the native nucleocapsid polypeptide is a highly specific assay which can detect as little as 0.1 ng of nucleocapsid antigen in a 10⁶-fold excess of cellular protein. As such, the assay is promising as a diagnostic and experimental tool.
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REFERENCES


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