Measles Virus-Specific Antibody Levels in Individuals in Argentina Who Received a One-Dose Vaccine

Marcelo H. Argüelles,1,2 Mariana L. Orellana,1,3 Alejandro A. Castello,1 Guillermo A. Villegas,4 Matilde Masini,1 Alejandra L. Belizán,1,3 Silvia González Ayala,5 Osmar D. Vera,1 and Graciela Glikmann1∗

Laboratorio de Inmunología y Virología, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes,1 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),2 Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT),3 and Centro de Virología Animal (CEVAN),4 Buenos Aires, and Servicio de Infectología, Hospital de Niños Sor María Ludovica, La Plata,5 Argentina

Received 17 May 2005/Returned for modification 4 January 2006/Accepted 8 June 2006

In spite of active measles virus (MV) vaccination strategies, reemergence continues to occur, impairing global eradication programs. The immune status against measles was evaluated in 350 vaccinated healthy Argentine children and teenagers who received a single dose of the MV Schwarz strain Lirugen vaccine (Aventis Pasteur). Sera were assessed for immunoglobulin G (IgG) antibodies by a commercial enzyme immunoassay (EIA) (Enzygnost; Behring), an in-house EIA, and neutralization EIA. Results obtained with these methods showed a marked decline in IgG level with increasing age. At 1 to 4 years of age, 84% of children had IgG antibodies above 200 mIU/ml, conventionally accepted as protective levels, whereas only 32% of older children and teenagers had antibody levels exceeding 200 mIU/ml. Moreover, the MV IgG content in the teenage group was significantly lower than the IgG antibody level of the group of younger children (P < 0.0001). In contrast, screening for IgG antibody levels to inactivated tetanus vaccine showed that, on average, 80% of this population was fully protected and that this high level of protection remained through the teenage years. This study suggests that within this population a considerable proportion of individuals had low measles antibody levels that may be insufficient to protect against reinfections or clinical disease.

In the early 1960s, the advent of a live attenuated measles virus (MV) vaccine dramatically reduced the incidence of measles in many parts of the world, including developed (12, 16, 20, 29, 30, 35, 49, 52) and developing (28, 58) countries. In some developing countries, fatality rates for measles can still be as high as 15%, causing about 770,000 annual deaths among infants and children, and this is probably due to lack of vaccination of many individuals in the population.

Elimination of MV requires the continued commitment to increase vaccination coverage levels, the genetic analysis of circulating strains, and serosurveys of vaccinated individuals to establish the population at risk of contracting the infection. In this context, it is very important that reliable and sensitive laboratory methods are used to accurately determine the antibody level and protection achieved after vaccination and the level of antibodies that persists in those who were previously vaccinated.

Despite active vaccination strategies, reemergence or resurgence of MV continues to occur, impairing elimination programs. The occurrence of several measles outbreaks in highly immunized populations (5, 34, 40, 51) has focused attention on vaccine efficacy and the durability of vaccine-induced immunity. It is likely that many factors contribute to the presence of susceptible individuals among highly vaccinated populations. These include failure to seroconvert and decline of immunity with time after vaccination (19, 37). Other important factors that might influence the immune response comprise the age at the time of vaccination (27, 33), the number of doses, and the strain included in the vaccine (18, 23, 28).

High vaccination coverage with a single-dose regimen has been unsuccessful in eliminating measles. Two-dose schedules have been implemented in many countries where measles has been eliminated (9, 46). The second dose provides an opportunity to vaccinate children who did not receive the first dose or to protect children with primary vaccine failure.

In most developed countries, the immunization schedules have relied on the use of trivalent formulations for immunization against measles, mumps, and rubella, which contain different combinations of viral strains and/or viral doses (18, 23). In the Argentine Republic, the official schedule for MV vaccination until 1998 consisted of a single dose of MV Schwarz strain Lirugen vaccine (Aventis Pasteur) administered at 12 months of age.

The aim of the present study was twofold. The first goal was to evaluate the levels of measles immunoglobulin G (IgG) antibodies in one-dose-vaccinated children and teenagers in suburban districts of Buenos Aires province, Argentina. This retrospective study was conducted since evaluation of measles-specific IgG antibody levels after vaccination had never been performed in Argentina in spite of active vaccination since 1969. The second aim was to develop and evaluate alternative in-house methods for detection of MV antibodies, comparing their performances with that of a certified commercial assay. The development of such low-cost methodology with performance comparable to that of more-expensive commercial kits

∗ Corresponding author. Mailing address: Laboratorio de Inmunología y Virología, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Sáenz Peña 352, Bernal (B1876BXD), Buenos Aires, Argentina. Phone: 54 11 4365 7100, ext. 163. Fax: 54 11 4365 7132. E-mail: gglikman@unq.edu.ar.
is very important for laboratories with a restricted budget. These assays will be useful in future studies involving large numbers of samples taken from individuals vaccinated with a different regimen.

**MATERIALS AND METHODS**

**Study population.** The samples were obtained in 1997 (February to October). Healthy children and teenagers from 12 months to 18 years old living in suburban southern districts (Benzaguel, San Roque, Saavedra, and Polvoradas) of Buenos Aires province were randomly selected using a provincial database. A total of 350 individuals were enrolled in the study. Informed parental consent for sample collection was obtained for all individuals. Blood samples were taken by venipuncture, and sera were immediately separated and kept frozen at −20°C. A questionnaire concerning measles vaccination, measles exposure, and history of natural infection was completed for each individual. The date of vaccination and the type of vaccine given were obtained from each child’s vaccination card. An individual was included if she or he had received a single dose of measles live virus vaccine between 12 and 24 months of age. The Ethics Committee of the Hospital de Niños Sor María Ludovica, La Plata, Argentina, approved the study protocol.

**Measles vaccine, measles virus, and polyclonal measles antibodies.** (i) **Measles vaccine.** The population under study received a single dose of the measles live virus vaccine (Aventis Pasteur). According to the manufacturer’s description, the measles vaccine has a minimum of 1,000 50% tissue culture infective doses per ampoule. The Argentine Public Health Ministry distributed the vaccine to all regional vaccination centers. (ii) **Measles virus.** The Edmonston strain of measles virus (VR24; ATCC, Manassas, VA) was cultivated in monolayers of Vero cells according to standard procedures. Virus suspensions containing 10^5 to 10^6 PFU/ml were subsequently clarified by low-speed centrifugation, concentrated by differential centrifugation on a 30% (wt/wt) sucrose cushion at 100,000 g, quantified by the Bradford method (11), and used as the viral antigen for the in-house enzyme immunoassay (EIA). (iii) **Polyclonal measles antibodies.** Measles virus purified according to methods published elsewhere (25, 55) was used to prepare rabbit hyperimmune sera (57). Polyclonal rabbit IgG was affinity purified by protein G Sepharose 4 Fast Flow (Pharmacia, Uppsala, Sweden) chromatography according to the manufacturer’s recommendations and biotin (Sigma Chemical Co.) labeled by the N- hydroxysuccinimide derivative method as described elsewhere (25).

**Detection of human measles IgG antibodies.** (i) **In-house EIA.** Microtiter plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with measles virus antigens diluted to 10 µg/ml in 0.1 M bicarbonate buffer, pH 9.6. After this and the following steps, the plates were washed three times with washing solution, 3% phosphate-buffered saline (PBS), 0.5% NaN3, and 0.2% (wt/vol) Triton X-100. Fifty microliters of each serum sample diluted 1/100 in sample buffer (i.e., 1% [wt/vol] bovine serum albumin in washing solution) was added to the antigen-coated wells, and the plates were incubated at 37°C for 1 h. Peroxidase-conjugated anti-human IgG (DAKO A/S, Glostrup, Denmark) diluted 1/5,000 in sample buffer was added at 100 µl/well and incubated further for 1 h at 37°C. Bound antibodies and conjugates were then revealed with ortho-phenylenediamine (Sigma Chemical Co.), 30% H2O2, and citrate buffer, pH 5.0, at a ratio of mg/ml µl according to standard procedures. The optical density (OD) was measured at a wavelength of 490 nm (OD<sub>900</sub>) (Max Line TM enzyme-linked immunosorbent assay reader; Molecular Devices, Sunnyvale, CA). Each serum was simultaneously tested with corresponding standards and negative controls. The results were expressed as the value of the control antigen OD subtracted from the virus antigen OD of each serum sample. The enzyme-linked immunosorbent assay cutoff value was estimated as the mean OD obtained with 20 certified negative specimens plus 3 standard deviations. This control group of sera was taken from nonvaccinated healthy children at 1 year old without a past history of measles. Furthermore, these sera gave negative results when tested by Western blotting. The cutoff value obtained in this way was ≤0.2. Two positive-control sera taken from children 1 month after an acute episode of measles and two negative-control sera were always included in the test, with reproducible results. A standard reference serum provided by the WHO (Statens Seruminstit, Copenhagen, Denmark) was used to estimate the concentrations of MV IgG in international units per milliliter. This reference serum, diluted to contain 10 IU/ml, was then serially twofold diluted and included in each plate in order to generate a standard reference curve from which the CV-specific IgG content, in mIU/ml of each sample, was calculated. (ii) **Measles neutralization (NT) EIA.** The Edmonston strain of measles virus was grown in minimal essential medium (Sigma Chemical Co.) with 3% fetal calf serum (Gibco). Heat-inactivated (56°C for 30 min) human sera were diluted 1/10 in fresh medium, serially twofold diluted, added to wells (4 wells/dilution) of a 96-well plate (Nunc) at 50 µl/well, and mixed with an equal volume of freshly diluted measles virus containing 50 PFU. After incubation for 1 h at 37°C in a 5% CO2 incubator, Vero cells were added at 2 × 10^4 cells per well in 100 µl. The plates were incubated at 37°C in the presence of 5% CO2 for five additional days and inspected daily for cytopathic changes. After that, the medium was carefully removed and the infected cells were fixed with 90% (vol/vol) acetone diluted in distilled water for 10 min at −20°C. The plates were washed once with PBS, pH 7.4, and thereafter with EIA washing solution. A 1/50 dilution of biotinylated MV antibodies in EIA sample buffer (see above) was added to all wells at 50 µl/well. After a 1-h incubation at 37°C, the plates were rinsed as described before and a 1/1,000 dilution of avidin-peroxidase (DAKO A/S) conjugate was added to all wells and incubated at 37°C for 30 min. After the final wash, color development was carried out using 3,3′-diaminobenzidine (Sigma Chemical Co.) at 0.6 mg/ml (wt/vol) diluted in PBS, pH 7.4, with 1 µl/ml (vol/vol) 30% H2O2 for 15 min at room temperature, protected from light. Plates were inspected microscopically for dark-brown cytoplasmic inclusions. Neutralizing antibody titer was calculated as the highest dilution showing 50% reduction in colored cytoplasmic inclusions compared to results with control wells that contained virus without serum. The 50% endpoint was calculated by the Karber method. (iii) **Commercial EIA.** The commercial anti-measles virus-immunoglobulin G assay (Enzygnost; Behring, Marburg, Germany) was performed according to the manufacturer’s recommendations and used for additional testing of each serum sample. TT IgG antibodies. Detection of IgG antibodies to tetanus toxoid (TT) was performed as an indirect EIA with reagents kindly provided by Claus Koch (Statens Serum Institut, Copenhagen, Denmark) according to a previously described method (32). Briefly, TT at 742 LF/ml (where LF is the limit of flockulation) was diluted to 5 µg/ml in 0.1 M bicarbonate buffer, pH 9.6, and used for coating of microtiter plates (Nunc) overnight at 4°C. Washing solution, washing protocol, and sample buffer were otherwise the same as described above for measles IgG antibodies. Sera diluted 1/200 in sample buffer were added in 100-µl volumes to antigen-coated wells and incubated at 37°C for 1 h. After plates were washed, peroxidase-conjugated anti-human IgG (DAKO A/S) diluted 1/5,000 in sample buffer was added at 100 µl/well and incubated further for 1 h at 37°C. Bound antibodies and conjugates were then revealed with ortho-phenylenediamine as described above for the measles in-house EIA. Two positive-control standard sera included in the kit, containing 1.5 and 10.3 IU/ml, respectively, were serially twofold diluted and included on each plate. The concentration of IgG antibodies, in IU/ml, was estimated for each serum sample by extrapolation from the standard curve obtained with reference sera. Those serum samples exceeding the upper level were twofold diluted and retested.

**Statistical analysis.** The Student t-test for paired samples and the Wilcoxon rank sum test for independent samples were used to compare the differences in protective IgG levels to MV and TT in children (1 to 4 years old) and teenagers (10 to 18 years old), respectively. The t test was employed when the assumption of a normal distribution of samples was corroborated. Analysis of variance (ANOVA) was carried out to compare the mean values of MV protective IgG levels of individuals from eight different age groups, ranging from 1 to 18 years old. Statistical differences among the mean antibody levels of individuals from all age groups were evaluated by the Tukey method, with a 95% simultaneous confidence interval for specified linear combinations.

**RESULTS**

**Performance of measles antibody detection methods.** In the present study, three different methods were used to assess MV antibody levels in vaccinated children. The purpose of performing different methods was to evaluate the performance of two in-house methods (EIA and NT EIA) to determine their validity as low-cost alternatives to a certified commercial EIA (Enzygnost). The comparison of the in-house EIA with a certified com-
TABLE 1. Comparison of the Enzygnost EIA with the in-house EIA and the NT EIA

<table>
<thead>
<tr>
<th>Method</th>
<th>IgG level (mIU/ml) or neutralizing antibody titer</th>
<th>No. of samples with Enzygnost EIA result</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Agreement (%)</th>
<th>R² with Enzygnost EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-house EIA</td>
<td>&gt;200</td>
<td>306</td>
<td>98.4</td>
<td>100</td>
<td>88.6</td>
<td>98.6</td>
<td>0.815</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;200</td>
<td>5</td>
<td>100</td>
<td>100</td>
<td>88.6</td>
<td>98.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT EIA</td>
<td>≥80</td>
<td>295</td>
<td>96.1</td>
<td>90.7</td>
<td>76.5</td>
<td>95.4</td>
<td>0.725</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;80</td>
<td>12</td>
<td>97.8</td>
<td>87.3</td>
<td>96.1</td>
<td>95.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a A total of 350 serum samples with measles IgG Enzygnost EIA values ranging from 1 to 5,000 mIU/ml were tested for MV antibodies with the in-house EIA and NT EIA.

b IgG levels are given for the in-house EIA method, and neutralizing antibody titers are given for the NT EIA method.

c Percentages of samples showing agreement out of total number of samples yielding levels of MV IgG of >200 mIU/ml or <200 mIU/ml.

d The R² values were calculated on the basis of the best polynomial trend line (order 2).

Commercial kit (Enzygnost EIA) for all 350 serum samples gave an R² value of 0.815, which represents a satisfactory positive correlation between both assays. Both methods were compared with respect to the numbers of positive, negative, or discrepant results. Considering the Enzygnost EIA as a reference method, the sensitivity, specificity, and positive predictive value of the in-house EIA were in the range of 98.4 to 100%, demonstrating a good performance for this method (Table 1). However, only 5 out of 350 samples gave discrepant results when tested in parallel. These samples were positive only by the Enzygnost assay, although with low IgG concentration values (average, 350 mIU/ml; range, 240 to 560 mIU/ml). When the same specimens were tested by the in-house assay, the OD values obtained were either very close to or below the cutoff level and were therefore considered negative by this method. Nevertheless, in spite of this discrepancy, these five specimens were considered positive in view of the results obtained with the certified commercial method when antibody level was evaluated according to age (see below).

Comparison of the results obtained with the Enzygnost EIA and the NT EIA indicated that, in spite of satisfactory sensitivity and specificity values, the NT method gave an increased number of discrepant results. As can be seen from Table 1, a positive predictive value of 98.7% and a negative predictive value of 76.5% were calculated for this method, thus indicating a less satisfactory performance for the NT method. The cutoff level of the NT EIA titer was 10, which corresponds to the lowest antibody level detected by this method under our assay conditions. Using the international standard serum diluted to 200 mIU/ml, the NT EIA gave a titer of 80. The correlation between the titers given by the measles NT EIA with respect to the IgG concentration values in mIU/ml detected by the Enzygnost EIA was an R² value of 0.725, which is adequate although lower than the correlation obtained between the in-house and commercial EIAs (Table 1).

MV IgG levels according to age. When the individuals were grouped according to increasing age, a marked decline in IgG levels was observed for the older groups. Although serum samples were screened by the three methods described above, the results described in this section were obtained with the Enzygnost EIA. At least 80% of individuals belonging to the first six age groups shown in Fig. 1 had IgG levels above 200 mIU/ml. The next group, which included individuals from 10 to 12 years old, showed a decrease in the level of IgG antibodies (68.75%). However, a measles IgG above 200 mIU/ml was found in only 32.26% of teenagers (13 to 18 years old).

ANOVA demonstrated significant differences (P < 0.00001) in antibody levels present in children (1 to 4 years old) compared to levels present in teenagers (10 to 18 years of age). Although a continuous decline in IgG levels with increasing age was observed, no significant differences (ANOVA, P > 0.1) were found between antibody levels detected in individuals from the first two age groups (Fig. 1).

MV and TT IgG levels in vaccinated individuals. IgG levels to MV and TT in vaccinated individuals grouped in two age categories, children (1 to 4 years of age) and teenagers (10 to 18 years old), were compared.

The measles IgG content in the teenage group was significantly lower than the IgG antibody level in the group of younger children (50.8% versus 84.4%, respectively) (Wilcoxon test, P < 0.0001). Conversely, comparison between TT IgG contents in the same groups did not show significant differences (Wilcoxon test, P > 0.1), since 73.4% of children and 87.3% of teenagers had adequate IgG levels against TT.

These results indicate that, on average, a considerable proportion (49.2%) of teenagers (10 to 18 years old) had MV IgG levels below 200 mIU/ml. Meanwhile, the TT component of the trivalent diphtheria, tetanus, and pertussis vaccine elicited an appropriate individual immune response, which was maintained at older ages (data not shown).

DISCUSSION

This serological study examined the prevalence of antibodies to measles in vaccinated children and teenagers following the national measles vaccination program in Argentina before 1998, which used a one-dose regimen.

Both cellular and humoral responses (7, 54–56) are involved in immunity to MV. Although passive administration of antibodies protects an individual against natural infection even in the absence of a cellular response (2), the importance of cellular immunity in clearing infection has been well established for immunodeficient children (38). The relevance of serum antibodies to susceptibility or immunity remains an unsettled issue. For this reason, the limit of 200 mIU/ml, although conventionally accepted as a protective level (3, 6, 13, 17, 22, 31, 35, 41, 48, 50), should not be taken as an absolute. However,
determination of MV neutralizing antibodies closely correlates with protection from infection and disease (14).

Antibodies have traditionally been used as markers of vaccine-induced immunity because they are easier to measure and quantify than cell-mediated immunity (38). Monitoring of humoral immunity is based upon detection of specific IgG antibodies, assessed mainly by immunoassays using the whole virus or recombinant proteins (10, 12, 20, 26, 49). Neutralizing MV antibodies can be measured in vitro by standard neutralization test and more accurately by its enhanced version, the plaque reduction neutralization test (3, 14, 47). However, the plaque reduction neutralization test is currently carried out in only a few laboratories in the world because of technical difficulties. The performances of the in-house methods described herein (EIA and NT EIA) were quite satisfactory with respect to sensitivity and specificity compared to a certified commercial EIA, indicating that these methods can be used as alternatives to estimate IgG levels in vaccinated populations. Although the discrepancies between these methods were low (1.43% for the in-house EIA and 4.6% for the NT EIA), samples considered negative by in-house methods were retested with a certified method to avoid misclassification of the specimens being evaluated.

Approximately 84% of children included in this study had measles antibody levels exceeding those values (>200 mIU/ml) that are believed to confer protection (3, 6, 13, 17, 22, 31, 35, 41, 48, 50), whereas only 32% of teenagers showed IgG levels above 200 mIU/ml. Accordingly, the antibody levels to MV, expressed in mIU of IgG, showed a marked decrease with increasing age. These results are in agreement with previous observations (15, 19, 37) showing that vaccination-induced MV antibodies decline with time in the absence of natural booster infections or a second dose of vaccine. This indicates that a single-dose strategy at an early age results in IgG levels that vanish in adolescence, making this group at high risk of acquiring a milder or subclinical form of the infection (36, 45). Although revaccination is advisable for all of those individuals, it should be taken into account that priming of T-cell immunity may have resulted because of vaccination, but very little neutralizing antibody could have been made (21). However, upon exposure to wild-type measles those individuals may have mounted a memory response, sparing clinical disease (1).

On the contrary, antibody responses to the TT component of the trivalent vaccine were satisfactory in both 1- to 4-year-old children and teenagers. This difference might be explained by the fact that MV vaccine was given as a single dose, in contrast to the diphtheria, tetanus, and pertussis vaccine, which was given in four repeated doses according to the Argentine schedule.

Another factor that perhaps could have contributed to these results is the characteristic features of these vaccines. Attenuated MV vaccines are easily inactivated when the cold chain required for vaccine maintenance is discontinued (4), whereas the inactivated TT component of trivalent vaccines has a remarkable stability independent of storage temperature. Interruption of cold-chain facilities might represent a problem in temperate regions of low socioeconomic income, like suburban Buenos Aires neighborhoods (4).

Another important issue to consider is primary vaccine failure, which can increase the number of susceptible individuals in vaccinated populations (43). Primary vaccine failures or failure to seroconvert may account for variable proportions of children without detectable MV antibodies after vaccination. Our data indicate that about 16% of children from 1 to 4 years old might have undergone primary vaccine failures, since antibodies were not detected after vaccination. Failures to seroconvert have been shown to be dependent on several factors, including the number of doses, the strain included in the vac-

FIG. 1. Measles IgG levels, calculated in mIU/ml using the Enzygnost assay (Behring), are depicted for each age group, with the mean value (horizontal line in each gray box) and corresponding range (vertical bars) given. Values either below or above the range limits are indicated as filled circles. Each age category comprises, on average, 32 individuals (range, 31 to 32).
cine used, the geographical region, and the age of first vaccination because of persistence of maternal antibodies (8, 44). However, a previous report showed that as many as 80% of Argentine infants were susceptible to MV infection at 9 months (39), indicating that they did not have maternally derived measles antibodies by the age of vaccination.

The susceptibility profile for a particular country can be estimated from vaccination coverage and disease incidence data, but the most direct evidence comes from seroprevalence studies (24).

Although the conclusions outlined here were based on a restricted number of individuals, they have provided some indication of the prevalence of antibody levels in vaccinated individuals with a single-dose monovalent vaccination schedule. In 1998, an epidemic outbreak of measles occurred in Argentina, where an 80- to 100-fold increase in the number of reported cases was registered (42). Although no direct link can be drawn between the seroprevalence data from 1997 reported here and that outbreak, the absence of new epidemics in the country for the past 8 years could be related to the implementation of the two-dose schedule after 1998. A second dose with the trivalent formulation MMR II (Merrick, Sharp & Dome) has been included at the age of 6 years. This vaccine has been used here and that outbreak, the absence of new epidemics in thecountry for the past 8 years could be related to the implementation of the two-dose schedule after 1998. A second dose with the trivalent formulation MMR II (Merrick, Sharp & Dome) has been included at the age of 6 years. This vaccine has been used for the first immunization at 12 months, replacing the monovalent vaccine. Including a second dose will not only provide a protective titer for the first immunization at 12 months, replacing the monovalent vaccine. Including a second dose will not only provide a protective titer, it will boost the titers in already-vaccinated individuals.

The information obtained from the present study may be useful in future years to evaluate and compare immune statuses achieved with the two-dose regimen introduced in Argentina in 1998.

ACKNOWLEDGMENTS

We are grateful to Claus Koch (Immunology Department, Statens Seruminstitut, Copenhagen, Denmark) for supplying us with the EIA kit and reference sera for determination of TT IgG antibodies. We thank Carl H. Mordhorst (Virology Department, Statens Seruminstitut) for providing us with MV international reference serum. The Enzymuntest EIA commercial kits were a kind gift from Aventis-bio-Merieux, Argentina.

This work was supported by grants PICT 3856 and PICT 12252 from Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT).

REFERENCES