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# Observational study on immune response to yellow fever and measles vaccines in 9 to 15-month old children. Is it necessary to wait 4 weeks between two live attenuated vaccines?

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ABSTRACT

*Background:* The use of 2 live attenuated vaccines (LAV) is recommended to be simultaneous or after an interval of at least four weeks between injections. The primary objective of this study was to compare the humoral response to yellow fever (YF) and measles vaccines among children vaccinated against these two diseases, either simultaneously or separated by an interval of 7–28 days.
*Subjects and methods:* A prospective, multicenter observational study was conducted among children aged 9–15 months. The primary endpoint was the occurrence of positive yellow fever antibodies after YF vaccine by estimating the titers of neutralizing antibodies from venous blood samples. Children vaccinated against YF 7–28 days after receiving the vaccine against measles (test group) were compared with children vaccinated the same day against these two diseases (referent group).
*Results:* Analysis was performed on 284 children. Of them, fifty-four belonged to the test group. Measles serology was positive in 91.7% of children. Neutralizing antibodies against YF were detected in 90.7% of the test group and 92.9 of the referent group ($p$=0.6). In addition, quantitative analysis of the immune response did not show a lower response to YF vaccination when it took place 1–28 days after measles vaccination.
*Discussion:* In 1965, Petralli showed a lower response to the smallpox vaccine when injected 4–20 days after measles vaccination. Since then, recommendations are to observe an interval of four weeks between LAV not injected on the same day. Other published studies failed to show a significant difference in the immune response to a LAV injected 1–28 days after another LAV. These results suggest that the usual recommendations for immunization with two LAV may not be correct.
*Conclusion:* In low income countries, the current policy should be re-evaluated. This re-evaluation should also be applied to travelers to yellow fever endemic countries.


## 1. Introduction

Yellow fever (YF) can be prevented by a 17D strain live attenuated vaccine (LAV). After vaccination, the neutralizing antibody response is obtained in 80–95% of immunocompetent subjects [1–6] and is considered protective from 10 days after vaccination [7] for several decades [8,9].

The YF vaccine is well tolerated [10,11]. Rare cases of the viscerotropic form of the disease following vaccination have been described [12–16] and groups at risk identified [17]. Except during epidemics, the vaccine is not recommended before 9 months. It is contraindicated for below 6 month children, in case of egg allergy [18] and in severely immunocompromised subjects [19,20].

In Senegal, the YF vaccine is administered as part of the expanded program on immunization (EPI) to 9 month-old children simultaneously with the first dose of monovalent measles vaccine.

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In French Guiana, the YF vaccine is compulsory at the age of 12 months.

Measles can also be prevented by a LAV, administered standalone or in combination with vaccines against rubella, mumps and rubella or varicella. In Africa, widespread vaccination against measles has significantly dropped mortality rates in countries where the EPI has been strengthened [21,22].

Two different LAV should be administered simultaneously or at least after 4 weeks interval [23]. This recommendation is based on the assumption that the interferon response following the injection of the first LAV could reduce the response to the second LAV [24,25]. In some circumstances, this recommendation cannot be followed: vaccine stock shortages, unplanned travel to YF endemic areas for example.

In Brazil, a study evaluating the effectiveness of a YF vaccine administered 1–28 days after measles vaccination showed no difference on antibodies titers [26].

The present study aimed to compare the humoral response to YF and measles vaccines in children aged from 9 to 15 months, routinely vaccinated against these two diseases, either with an interval of 7–28 days (Group1 = test group) or simultaneously (Group2 = referent group).

## 2. Subjects and methods

### 2.1. Study design

This prospective, multicenter (French Guiana, Senegal) observational study compared groups of children vaccinated against YF and measles either simultaneously or at an interval of 7–28 days in the context of every day practice.

All children 9–15 months old, resident in Senegal or French Guiana not vaccinated against YF and consent form signed by the legal guardian were eligible.

The day of immunization against YF was the day of inclusion in the study. The children who, for any reason, had been vaccinated against measles 7–28 days before inclusion were assigned to test group. Children vaccinated the same day against YF and measles were assigned to referent group. Exclusion criteria were a contraindication to one of the vaccines used, a known chronic disease or an acute infection present on the day of vaccination.

The main endpoint was the YF neutralizing antibody titer and the comparison of antibody response in the two groups of children (correlate of protection = titer $\geq$ 1:10 for YF). These titrations studies were performed on venous blood samples taken between 28 and 60 days after YF vaccination. The occurrence of adverse events following YF vaccination was also documented.

### 2.2. Study conduct

The study was carried out at Cayenne (French Guiana) and Dakar (Senegal). The vaccines used for YF vaccination were Stamaril® (Sanofi Pasteur, France) in French Guiana and the YF vaccine produced by the Institut Pasteur in Dakar (Vaccin Amaril Stabilise®) in Senegal. For measles, trivalent vaccines against measles, mumps and rubella (Priorix® or M-M-Rvaxpro®) were used in French Guiana. A monovalent vaccine (Rouvax®) was used in Senegal.

For every child included, two visits were planned: one at inclusion (V1), the day of vaccination with the YF vaccine, and a final follow-up visit 28–60 days after vaccination (V2). During V1, the physician had to check for the absence of exclusion criteria and perform anthropometric measurements. At V2, he reviewed the occurrence of adverse events and took a sample of venous blood for the YF and measles serologies. 1–2 ml of whole blood were collected. Serological analysis were performed by the Virology units of the Instituts Pasteur in Dakar and in French Guiana.

### 2.3. Serological tests

The two laboratories used standardized plaque reduction neutralization test (PRNT) protocol and interpretation criteria. PRNT represents a sensitive, reproducible and functional method to measure YF neutralizing antibodies [27,28]. Briefly, employing a cell culture and carboxymethyl cellulose overlay procedure, a defined virus test dose (d) of $10^3$ plaque-forming units (PFU)/ml was used to prepare the following dilution range of virus: d50, d70, d90, d95 and d99. The neutralizing capacity was calculated from virus test dose (d) yielding 30 plaque-forming units of virus (PFUVs) and the cut-off for protection was defined at d90 virus dilution. First we tested capability of diluted serum (1:10) to neutralize YFV dilution d90. In a second step we determined, using two-fold serum dilutions (from 1:20 to 1:640), the last dilution of serum that neutralized YFV dilution d90. This limiting dilution represents the antibody concentration causing a 90% reduction. Antibody results for YFV PRNT were expressed quantitatively using the titration of neutralizing antibodies.

For measles, a commercially available indirect ELISA kit (Captia™ Measles IgG, TRINITY Biotech, Bray, Ireland) was used to detect anti-measles IgG antibodies.

Antibody results for MV and YFV were expressed qualitatively (positive/negative) for statistical analysis.

### 2.4. Adverse events

Adverse events were searched at V2, using a standardized questionnaire (fever, nausea, pain at injection site, etc.). Serious adverse event, linked or not to the vaccination, were reported using a standardized form of the French National Agency for the Safety of Medications and Health Products – ANSM) [23]. For Senegal, serious adverse events were reported to the Department of Pharmacies and Laboratories and the National Committee on Health Research Ethics. In French Guiana, they were reported to the Regional Center for Drug Safety Monitoring.

### 2.5. Sample size calculation

The sample size calculation was based on YFV seroconversion rate of 95% in the referent group and of 80% in the test group. Based on the ratio Group1/Group2 = 1/5, the total number of children to include was estimated at 270 (45 for Group1 and 225 for Group2), for a power $1 - \beta = 80\%$.

Expecting a proportion of subjects lost to follow up of 20%, 60 children in Group1 and 280 children in Group2 had to be included.

### 2.6. Processing and statistical analysis of data

Data were recorded on a standardized case report form then entered in computerized database using Epi-Info™ 3.5.1 software (CDC, Atlanta, USA). Data analysis was performed using Stata®10 software. The Chi-2 test or Fisher's exact test were used to compare qualitative variables. Student's *t*-test or the Mann–Whitney test were used to compare quantitative variables. Univariate and multivariate models (logistic regression) were used to identify covariates associated with a negative response to the YFV measured by PRNT. For the multivariate analysis, a step-by-step backward method was used. Nested models were compared using the likelihood ratio test. The adequacy of the models was measured using the Hosmer–Lemeshow test.

Subgroups analyses were performed. First, the children of the test group were divided into two subgroups: children vaccinated

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