Five accelerated schedules for the tick-borne encephalitis vaccine FSME-Immun® in last-minute travellers: an open-label, single-centre, randomized controlled pilot trial.

Running title: Accelerated schedules for the tick-borne encephalitis vaccination

Nicole Berens-Riha*, PhD, Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

Petra Andries, BSc, Centre for Infectious Diseases, Queen Astrid Military Hospital, Brussels, Belgium.

Annelies Aerssens, MD, Department of Internal Medicine and Infectious Diseases, Ghent University Hospital, Belgium.

Quentin Ledure, PhD, Hospital Pharmacy, Queen Astrid Military Hospital, Brussels, Belgium.

Yolien Vanderbeken, BSc, Clinical Laboratory, Queen Astrid Military Hospital, Brussels, Belgium.

Leo Heyndrickx, MSc, Virology Laboratory, Institute of Tropical Medicine, Antwerp, Belgium.

Els Genbrugge, MSc, Clinical Trials Unit, Institute of Tropical Medicine, Antwerp, Belgium.

Achilleas Tsoumanis, MSc Clinical Trials Unit, Institute of Tropical Medicine, Antwerp, Belgium.

Yven Van Herrewege, PhD, Clinical Trials Unit, Institute of Tropical Medicine, Antwerp, Belgium.

Kevin K. Ariën, PhD, Virology Laboratory, Institute of Tropical Medicine, Antwerp, Belgium; Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.

Martine Van Innis, PharmD, Hospital Pharmacy, Queen Astrid Military Hospital, Brussels, Belgium.

Peter Vanbrabant, BSc, Centre for Infectious Diseases, Queen Astrid Military Hospital, Brussels, Belgium.

Patrick Soentjens, PhD, Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium, and Centre for Infectious Diseases, Queen Astrid Military Hospital, Brussels, Belgium.

FASTBEPROTECT Research Team

FASTBEPROTECT Research Team: An Spiessens[2], Hanne Landuyt[5], Emeline de Viron[5], Kristy Zaman[2], Eric Dooms[2], Sarah Djebara[2], Katrijn Ghoos[2], Johan Michiels[3], Betty Willems[3], Jozefien Buyze[5], Benjamin Damanet[2], Katrien De Koninck[2], Mony Hing[2], Annelies De Hondt[5]

[1] Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium.

[2] Centre for Infectious Diseases, Queen Astrid Military Hospital, Brussels, Belgium.

[3] Virology Unit, Institute of Tropical Medicine, Antwerp, Belgium.

[4] Department of Internal Medicine and Infectious Diseases, Ghent University Hospital, Belgium.

[5] Clinical Trials Unit, Institute of Tropical Medicine, Antwerp, Belgium.

[6] Hospital Pharmacy, Queen Astrid Military Hospital, Brussels, Belgium.

[7] Laboratory, Queen Astrid Military Hospital, Brussels, Belgium.

[8] Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.

***Corresponding author:**

Nicole Berens-Riha

Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

nberens@itg.be

Preliminary results of the PRNT90 analysis only were presented as poster at NECTM 2022 (prize for best poster presentation).

Abstract

Background: The purpose of this exploratory study was to evaluate different accelerated tick-borne encephalitis (TBE) vaccine schedules for last-minute travellers.

Methods: In a single-centre, open-label pilot study, 77 TBE-naïve Belgian soldiers were randomized to one of the following five schedules with FSME-Immun®: group 1 ("classical accelerated" schedule) received one intramuscular (IM) dose at day 0 and day 14, group 2 two IM doses at day 0, group 3 two intradermal (ID) doses at day 0, group 4 two ID doses at day 0 and day 7, group 5 two ID doses at day 0 and day 14. The last dose(s) of the primary vaccination scheme were given after one year: IM (1 dose) or ID (2 doses). TBE virus neutralizing antibodies were measured in a plaque reduction neutralization test (PRNT90 and 50) at day 0, 14, 21, 28, month 3, 6, 12, and 12+21 days. Seropositivity was defined as neutralizing antibody titres ≥ 10.

Results: The median age was 19-19.5 years in each group.

Median time-to-seropositivity up to day 28 was shortest for PRNT90 in ID-group 4 and for PRNT50 in all ID groups. Seroconversion until day 28 peaked highest for PRNT90 in ID-group 4 (79%) and for PRNT50 in ID-groups 4 and 5 (both 100%). Seropositivity after the last vaccination after 12 months was high in all groups.Previous yellow fever vaccination was reported in 16% and associated with lower GMTs of TBE-specific antibodies at all time points. The vaccine was generally well tolerated. However, mild to moderate local reactions occurred in 73-100% of ID compared to 0-38% of IM vaccinations, persistent discolouration was observed in nine ID vaccinated individuals.

Conclusion: The accelerated two-visit ID schedules might offer a better immunological alternative to the recommended classical accelerated IM schedule but an aluminium-free vaccine would preferable.

1. Background

Tick-borne encephalitis (TBE) is a viral disease mainly transmitted by the bite of an infected tick (Ixodes sp.) and endemic in Asia and Central and Eastern Europe. Every year 10,000 to 15,000 new cases are reported with increasing numbers. [1] One reason might be the growing extent of tick habitats due to climate change and an increase in exposure prone activities. [2]

The tick-borne encephalitis virus (TBEV) consists of positive single-stranded RNA and belongs to the *Flaviviridae* family, genus Flavivirus. It enters through the skin via the tick saliva that contains components enhancing TBEV dissemination. The TBE aetiopathology comprises two phases. During the first viraemic phase the patient suffers from non-specific symptoms such as fever, headache, fatigue, myalgia, nausea and vomiting. [3] In one to two thirds of symptomatic patients the virus crosses the blood brain barrier (BBB) resulting in a second neurological phase with symptoms as meningitis or neurological focal forms. [4] The case fatality rate reaches 2-3% in Siberia, where sporadically haemorrhagic forms were described. [5] Serological surveys on the other hand indicate asymptomatic courses accounting for 70 to 98% of all infections. Approximately, only one in every 100-300 tick bites results in symptomatic infection. [6]

Analysis of TBEV-specific IgM and IgG antibodies by enzyme-linked immunosorbent assay (ELISA) is performed in routine diagnostics but cross-reactivity due to infection with or vaccination against other flaviviruses like West Nile (WN), Japanese encephalitis (JE), dengue or yellow fever (YF) can result in misinterpretation of results. [7] A former YF vaccination might even impair the efficacy of TBE vaccination. [8] The most sensitive method is the plaque reduction neutralization test (PRNT) which is only available in specialised laboratories. [1]

The lack of a standard effective treatment emphasizes the importance of disease prevention via vaccines. [9] Available vaccines are safe and effective. FSME-IMMUN® (Pfizer, Neudörfl strain) was first approved in 1976 for endemic regions. Encepur® (GlaxoSmithKline, K23 strain)

was introduced in 1991 in Germany, others followed. [4,7] For FSME-IMMUN®, the virus is produced in primary chicken embryo fibroblast cells (PCECs) and adsorbed on hydrated aluminium hydroxide. [10]

Since 2011, the World Health Organization (WHO) recommends vaccination for travellers who plan outdoor activities during their travel in endemic regions. [11] The standard administration schedule consists of two intramuscular (IM) doses given one to seven months apart and a third dose one year later, it does not meet the needs of last-minute travellers including soldiers who are sent on missions on short term notice. The accelerated IM schedule with two vaccinations at day 0 and 14 is approved for FSME-IMMUN® and Encepur® and recommended for rapid immunisation. [12] The purpose of this pilot study was to evaluate different accelerated TBE vaccination schedules by reducing the number of visits and intervals. We parallelly investigated the intradermal (ID) administration route as lower volumes could consequently reduce the vaccine costs in the case of group or mass vaccinations

2. Methods

Study design and objectives

This study was an exploratory single-centre open-label randomized controlled trial with FSME-IMMUN® in TBE-naïve soldiers in a non-endemic area. Group 1 (classical accelerated schedule) received one intramuscular (IM) dose at day 0 and at day 14 (thereafter "3^{1}IM"), group 2 two IM doses at day 0 ("$2^{2,1}$IM"), group 3 two intradermal (ID) doses at day 0 ("2^{2}ID"), group 4 two ID doses at day 0 and at day 7 ("3^{2}ID7"), and group 5 two ID doses at day 0 and at day 14 ("3^{2}ID14"). A last vaccination to finalise the primary schedule was given after one year IM or ID. (Figure 1)

The primary objective of this study was to estimate the median time to seroconversion of the different groups based on immunogenicity data up to 28 days after the first dose. Seroconversion was defined as neutralizing antibodies ≥10 and was determined by the plaque reduction neutralization test 90 (PRNT90) and PRNT50 (sensitivity analysis). Further the proportion of subjects with seroconversion at every visit for each vaccination regimen and the geometric mean titres (GMTs) at all visits in all groups were estimated.

Solicited and unsolicited adverse events (AEs) were recorded after each vaccination session for seven days, and serious adverse events (SAE) were reported for 14 days after vaccination. An amendment for a further follow-up of vaccine-related local reactions was added.

Study site, subjects and inclusion criteria

The study was conducted at the Centre of Infectious Diseases, ID_4C, in the Military Hospital Queen Astrid (MHQA), Brussels, Belgium, between May 2019 and December 2021. Participants were recruited in the Belgian defence personnel. Randomization was performed at the enrolment visit using a scratch list specifying the study group. The inclusion criteria were defined as age between 18 to 60 years, willingness to provide an informed consent, and use of safe contraception methods during the study. Seropositive subjects (tested during the screening visit) or with a known allergy to one of the components of the vaccine were excluded from the study. Further exclusion criteria were: immunosuppression, intake of immune-depressant or -stimulant medication, pregnancy or active child wish, planned deployment to TBE endemic regions or a yellow fever vaccination during the study period. Vaccinations with an inactivated vaccine within two weeks before or after each vaccination or with a live attenuated vaccine within one month before or after each vaccination were not allowed.

Laboratory procedures

The laboratory tests were performed at the Virology Laboratory of the Institute of Tropical Medicine. TBE virus neutralizing antibodies were measured in a plaque reduction neutralization test (PRNT). Six serial dilutions of heat-inactivated serum (1/10-1/320 in DMEM) were incubated during 1h (37°C, 7% CO2) with a pre-titrated amount of TBEV (Hypr strain). Sample-virus mixtures were added to previously (day -1) seeded A549 cells (adenocarcinomic human alveolar basal epithelial cells) in a 96-well plate and incubated during 2hr (37°C, 7% CO2) whereafter a CMC overlay was added. After a four day incubation period (37°C, 7% CO2) the supernatant was removed, cells treated with formaldehyde (30 minutes) and stained with Naphthalene Blue Black (NBB) solution (30 minutes). After removal of the NBB cells were rinsed with tap water and plaques counted. The Reed-Muench method was used to calculate the neutralizing antibody titre that reduced the number of infected wells by 50% (PRNT50) and 90% (PRNT90), which was used as a proxy for the neutralizing antibody concentration in the sample. All analyses of clinical trial samples were carried out in compliance with Good Clinical Laboratory Practice.

Vaccination procedure

The study vaccine FSME Immun® was stored in the fridge at a temperature between +2 and +8°C, as recommended by the manufacturer. It was brought to room temperature before administration. An intradermal dose consisted of 0.1 mL (or 1/5) of the vaccine vial. One full vial (0.5 mL) was administered for an IM vaccination. Double doses were given at different vaccination sites (for IM vaccination in the left and right deltoid muscle; for ID vaccination in the left and right forearm).

Statistical analysis

The primary and secondary objectives were analysed using both an Intention-to-Treat (ITT) and Per-Protocol (PP) population, with ITT as primary approach. Due to the COVID19 pandemic all participants had at least one out-of-window visit. It was therefore decided to exclude individual out-of-window visits in the PP population so that the subjects would not be excluded entirely from the PP analyses. The visits at month 6 and 12 were most affected by this, resulting in a lowsample size at these visits.

Safety analyses were performed using an all-patients-treated approach, including all participants who received at least a single vaccination.

Participants were censored at the first of the following events: day 28 after start of the primary vaccination, lost-to-follow-up or withdrawal. The median time to seropositivity with 95% confidence interval was estimated per schedule accompanied by a Kaplan-Meier plot. Three participants had a missing intermediate serology result an all other serology results (before and after missing result) were <10. Therefore, it was decided to impute the missing values as <10. The number and proportion of participants who were seropositive at each visit was estimated with a 95% Wilson confidence interval for the five vaccination regimens. The incidence of safety endpoints was estimated with 95% Wilson confidence interval for each group separately. Geometric mean titres were calculated with 95% CI for per group for each visit. Values <10 were replaced by 5 for this purpose.

Due to recent findings [8] that a former yellow fever vaccination might potentially influence the outcome of TBE vaccination, an exploratory analysis was performed. Antibody titres and the proportion of subjects with neutralizing antibodies ($\geq$ 10) were compared between those with and without previous yellow fever vaccination, pooled over the arms. For the antibody titres, the geometric means and their ratio were calculated with 95% confidence intervals (Cis). The p-value

was obtained by means of a t-test for lognormal data (PROC TTEST Procedure with dist. = lognormal). All analyses were repeated with the PRNT50 results for sensitivity purposes.
All analyses were carried out in SAS/STAT® 12.3 (SAS Institute Inc., USA).

Ethics and registration

Written informed consents were obtained at screening. The Institutional Review Board of the ITM, the Ethics Committee of University Hospital of Antwerp (UZA) and the Competent Authorities of Belgium (FAMPH) approved the trial. The study was carried out in compliance with the Declaration of Helsinki and according to the most recent Good Clinical Practice guidelines, it was registered in the EudraCT public registry as Eudra-CT 2019-000801-61.

3. Results

Demographics

Ninety-six Belgian soldiers were screened. Of these, 77 TBE-naïve participants were enrolled and completed the first dose (Day 0). To each of the five groups 15-16 participants were assigned. Sixty-seven (87%) participants completed the full vaccination schedule, four were lost to follow-up and six withdrew consent due to the local side effects after ID vaccination. (Suppl. table 1)

Participants were aged between 18 to 49 years. Median age was 19-19.5 years in each group. Male subjects were over-represented (83%) compared to female subjects (17%). (Suppl. table 2)

Serology PRNT90

All serology data presented refer to the ITT-analysis since there was no marked difference with the results from the PP analysis. Median time-to-seropositivity was shortest for 3^2ID7 with 14

days and for 3^2ID14 with 21 days. (Figure 2) Seropositivity was first observed at day 7 for 2^2ID and 3^2ID7. 3^2ID7 peaked highest and earliest in terms of percentage seropositivity at day 14 (78.6%) whereas the other ID groups peaked at day 21 (56.3%, 3^2ID14) and 28 (46.7%, 2^2ID) and the IM groups 3^1IM and $2^{1,2}$IM at day 28 with 53.3% and 25.0%, respectively. No group showed seroconversion of all participants before the last foreseen dose(s) of the primary vaccination schedule at month 12. Decline of detectable antibodies above the seropositivity threshold after day 28 was substantial in all groups. All but two participants were seroconverted after the last dose(s). (Table 1, Suppl. figure 1 and 2)

Serology PRNT50 (sensitivity analysis)

The PRNT50 analysis showed results similar to those with PRNT90 but the lower cut-off resulted in higher seropositivity levels in all groups. (Figure 2) Median time-to-seropositivity was short with 14 days in all ID groups. 3^2ID7 and 3^2ID14 showed 100.0% seroconversion at day 28 and 21, respectively. 2^2ID peaked with 80.0% at day 21 whereas 3^1IM and $2^{1,2}$IM peaked at day 28 with 66.7% and 56.3% only. Seropositivity was 100.0% in all groups after the last dose(s). (Table 1, Suppl. figure 1 and 2)

Geometric mean titres

All ID groups showed higher GMTs than the IM groups at day 14-28, with 3^2ID7 presenting the highest titres at day 14 (PRNT90: 16.4 (95% CI 8.39-32.2)) and day 21 (PRNT50: 50.8 (95% CI 27.1- 95.1). GMTs for both cut-offs were markedly higher 21 days after the third vaccination compared to results after the first two vaccinations. Vaccination schedules with three vaccination visits in total (3^1IM, 3^2ID7, 3^2ID14) showed higher titres than those with only two vaccination

visits, schedules with an interval of 14 days (3^1IM, 3^2ID14) developed highest titres. (Figure 3, Suppl. table 3)

Serology and previous yellow fever vaccination

Twelve (15.6%) of the 77 participants had a previous yellow fever (YF) vaccination. They showed at each visit lower geometric mean titres of neutralizing antibodies than YF naïve participants, significantly so at day 21 and 28, month 12 and 21 days later for both cut-offs (pooled analysis over all groups). (Table 2) The PRNT90 analysis showed two non-responders (female, 33 years, group 2 / male, 33 years, group 4) throughout the study who had a YF vaccination in the past. With the cut-off PRNT50, both were seropositive after the last vaccination with very low titres (10 and 12). YF vaccinations were given between 1994 and 2018, no specific pattern between antibody response and date of vaccination was observed. (Suppl. Figure 3) None of the vaccinees had a documented Japanese encephalitis vaccination. Other flavivirus infections were not actively asked. None of the participants had been deployed to a dengue fever endemic country but past private exposure to flaviviruses cannot be excluded.

Safety

The vaccine was generally well tolerated. Until day 28, any vaccine-related adverse events (AEs) including general symptoms were observed in 4 (26.7%), 0 (0%), 1 (6.7%), 2 (13.3%), and 2 (12.5%) participants for group 3^1IM, $2^{2,1}$IM, 2^2ID, 3^2ID7 and 3^2ID14, respectively. However, mild to moderate local reactions occurred in 100.0% of the ID groups compared to 0.0% to 37.5% in the IM groups after the first dose(s), with lower prevalence after the last dose(s). (Suppl. table 4A and 4B) Sequelae at injection site after ID administration were further followed up, nine of 46 (19.6%) ID participants developed persistent discolouration. Four participants are

still followed-up for more than three years and recently presented with reddish-brown spots (diameter 0.5-1 cm) at injection site. (Suppl. Figure 4) No vaccine-related SAEs were reported in the study.

4. Discussion

This non-commercial pilot study investigated five accelerated IM and ID schedules of TBE vaccination for last-minute travellers. The ID schedules showed in general a short median time-to-seropositivity and acceptable to excellent seroconversion until day 28 but local side effects were frequent and sometimes long-lasting discoloration was observed.

First reports about intradermal TBE vaccination in few individuals were published by an Austrian group in the 1980ies. A single multi-site ID vaccination of the same dose used for IM administration resulted in seroconversion of all vaccinees compared to only one third with single IM dose. [13, 14] Similarly, the ID groups showed better early immune responses than the IM groups in our study. ID administration seems to evoke fast seroconversion. All vaccinees with 3^2ID7 and 3^2ID14 were seropositive until day 28 and 21, respectively. The single-visit ID 2^2ID schedule peaked early at day 14 (80%). (PRNT50).

The classical accelerated IM schedule (3^2IM) resulted in a slow and insufficient antibody response, only half (PRNT90) to two third (PRNT50) of the participants in our study had seroconverted at day 28. Data are consistent compared to a published trial from the Czech Republic, in which seropositivity at day 21 after two IM doses (day 0, day 14) was achieved by only half of the participants (53%, PRNT50). [15]

The double dose IM (2 x 0.5 mL) at day 0 (group 2) showed a weaker response with only 25% (PRNT90) to 56.3% (PRNT50) of the participants having antibodies at day 28 and very low

GMTs in general. As in the Czech study one IM dose at day 0 resulted in 28-29% seropositivity, thus raises the question if a double dose has any benefit compared to a single dose.
After day 28, antibody levels waned in all groups, leading to the question if short-term seroconversion confers full mid-term protection. In any case, the last dose of the primary vaccination schedule showed an excellent booster effect.
Reduction of intervals and visit frequency seemed unfavourable when looking at the final GMTs: higher frequency of visits and longer intervals in between showed best results as expected (schedules with vaccinations at day 0, 14 and 365).

FSME Immun® was exclusively vaccinated in our study. Encepur® was used in the Czech study and showed similar results IM. Other new vaccines might evoke different immune responses or side effects. Although local reactions were mostly mild and short-termed, the frequency after ID administration was remarkable and for nine participants even persistent.
This is most likely attributable to the aluminium content of the vaccine. [16] Aluminium-free TBE vaccines might increase ID administration acceptance. The Russian aluminium-free vaccine Evervac® was found non-inferior to a commercial TBE vaccine in a phase I/II study. However, ID administration was not tested. [17]

Cross-reactions of the TBE virus with other flaviviruses are not broadly studied. TBEV is closest related to the Omsk haemorrhagic fever (OHF) virus that is endemic in Russia and can cause gastrointestinal or bleeding problems and sometimes encephalitis. [18] Flavivirus post-infection or post-immunization sera contain a subset of strongly cross-reactive antibodies that show low-affinity binding to virions, possibly resulting in low cross-reactive neutralizing activity and antibody binding enhancement. [19] A recent study by Bradt and colleagues showed that a pre-

existing yellow fever immunity could impair and modulate the antibody response to TBE vaccination. A TBE vaccination resulted in a strong boost of broadly flavivirus cross-reactive antibodies in YF pre-vaccinated participants but lower TBE neutralizing antibodies compared to YF-naïve participants. The effect was most pronounced after the second vaccination (day 28) and decreased over time, neutralizing antibodies equalled after the third vaccination. [8] These observations could be confirmed in our study. Due to the exploratory nature of our study, conclusions can only be drawn very cautiously but a previous YF vaccination corresponded with lower TBE GMTs at all visits. The time interval between the YF and the TBE vaccination varied highly and seemed to show no specific pattern concerning TBE antibody response. For both non/low-responders, a YF vaccination was documented. Possible factors influencing vaccine success like gender imparity or old age could be ruled out. [20-22] For European travellers, cross reactions between TBE, YF, JE and now dengue vaccinations are most relevant. Also imported dengue, West Nile or Zika virus infections will probably increase in the future and more dengue vaccines for travellers will come on the market. Interactions between flavivirus post-infection or post-vaccination antibodies need to be considered in future vaccine schedules.

The randomized controlled design and the overall good follow-up rate of 87% were a strength of the trial that was conducted by a team that has substantial experience in performing vaccine trials and appropriate intradermal injections. However, evident limitations of this trial were the low sample size, the explorative nature, and the fact that most participants were very young males being not representative for the whole population. The trial was not designed to make formal comparisons between the schedules; p-values from the exploratory analyses should be interpreted with care. The study was intended as a two-step approach: first an exploratory study with a low sample size to assess safety and plausible immunogenicity effect sizes without comparing the

groups formally. A full and formally powered non-inferiority trial would be planned afterwards. Although the immunogenicity data look promising, the persistent dermal discolouration after intradermal injection made us hesitate to vaccinate larger groups ID. To provide consultants with well-founded information for last-minute travellers, larger TBE vaccine studies with ID administration in a more diverse, gender-equal and especially older population are needed, but trial participants need to be informed about the potential persistent local discolouration if aluminium-containing TBE vaccines are to be used.

5. Conclusions

The accelerated TBE ID schedules 3^2ID7 and 3^2ID14 might offer a better immunological alternative for last-minute travellers at risk to the recommended classical accelerated IM schedule according to this exploratory pilot study. ID schedules with two visits before day 28 showed short median time-to-seropositivity of 14 days and 100% seroconversion until day 28 for PRNT50. Powered RCTs with a more diverse study population need to prove non-inferiority of the accelerated ID schedules compared to the standard IM schedule. However, the evaluation of aluminium-free alternatives would be preferable and their development should be encouraged.

Funding

The study was financed by a grant by the Royal Higher Institute for Defense Brussels ("HFM 18-1: ALL-IN-ONE: FASTBEPROTECT"). The Institute of Tropical Medicine of Antwerp (ITM) acted as sponsor of the study.

Potential conflicts of interest

None declared.

Declaration of Competing interests

No competing interests.

Data availability statement

Anonymised data can be shared in agreement with the ITM data sharing policy.

Acknowledgements

We thank the participants for their involvement in the study and all helping hands of the medical team of the Queen Astrid Military Hospital in Brussels during the vaccinations and administrative processes.

Authors' contribution

NBR was responsible for the data processing, participated in the data analysis and drafted the first manuscript. PA coordinated as responsible study nurse patient care, vaccinations and data entry. LH performed the neutralization tests. AA participated in study design and manuscript writing. EG and AT were responsible for the statistical analysis plan and performed the statistical analyses. QL coordinated and performed the vaccine handling. YV supervised sample collection and transport from the study site to the ITM laboratory. YVH was responsible for the data management and sponsoring tasks of the study. KKA coordinated and supervised the neutralization tests. MVI supervised the vaccine handling. PV participated in the trial and reviewed the manuscript. The FASTPROTECT research team was involved in designing and writing the protocol, statistical analysis, data and patient management. PS was responsible for the

design, the grant application and coordinated and supervised as PI the whole trial. All authors revised the manuscript.

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Figure 1. Study Design

Legend Figure 1. Study design of all five groups. Group 1 (3^1IM) with 3 intramuscular injections at day 0, 14 and month 12. Group 2 ($2^{2,1}$IM) with 3 intramuscular injections, two at day 0 and one at month 12. Group 3 (2^2ID) with 2 intradermal double injections at day 0 and month 12. Group 4 (3^2ID7) with 3 intradermal double injections at day 0, 7 and month 12. Group 5 (3^2ID14) with 3 intradermal double injections at day 0, 14 and month 12.

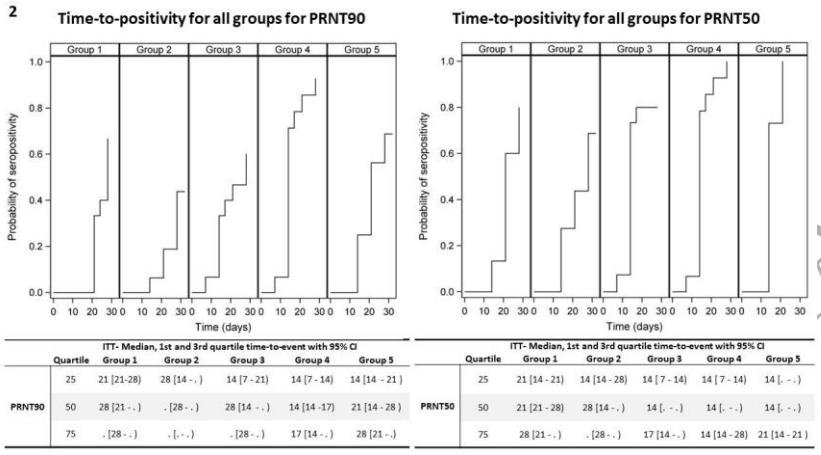
		Group	N	Dose	Screening	Day 0	Day 7	Day 14	Day 21	Day 28	Month 3	Month 6	Month 12	Month 12 + 21 d	Total volume
Blood sampling		All groups		10 mL / sample	✎		✎	✎	✎	✎	✎	✎	✎	✎	90 mL
Vaccination	IM	1: 3^1IM	15	**0,5** mL / injection		💉		💉					💉		1,5 mL
	IM	2: $2^{2,1}$IM	15	**0,5** mL / injection		💉💉							💉		1,5 mL
	ID	3: 2^2ID	15	**0,1** mL / injection		💉							💉		0,4 mL
	ID	4: 3^2ID7	15	**0,1** mL / injection		💉	💉						💉		0,6 mL
	ID	5: 3^2ID14	15	**0,1** mL / injection		💉		💉					💉		0,6 mL

💉 IM vaccination 💉 ID vaccination ✎ Blood sampling

Figure 2. Time to seropositivity for all groups for PRNT90 and PRNT50

Legend Figure 2. The Kaplan-Meier graphs show the time to seropositivity in days for the different vaccine schedules between day 0 and day 28. The curves describe the cumulative incidence of seroconversion (seropositivity) until day 28: every patient turned seropositive, stayed seropositive in this analysis, reconversion to seronegativity is not captured.

Group 1 (3^1IM), group 2 ($2^{2,1}$IM), group 3 (2^2ID), group 4 (3^2ID7), group 5 (3^2ID14), ITT analysis



ITT- Median, 1st and 3rd quartile time-to-event with 95% CI

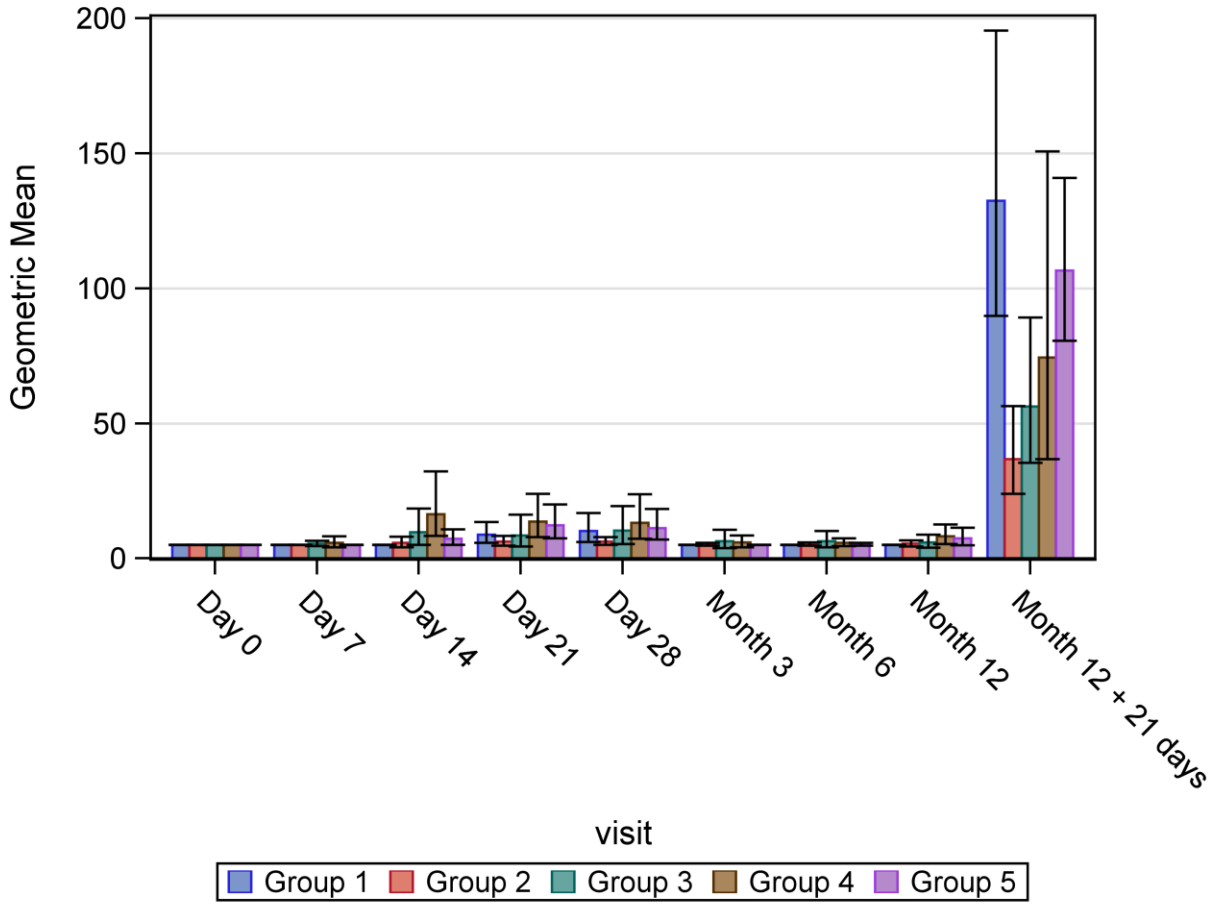
	Quartile	Group 1	Group 2	Group 3	Group 4	Group 5
PRNT90	25	21 [21-28)	28 [14 - .)	14 [7 - 21)	14 [7 - 14)	14 [14 - 21)
	50	28 [21 - .)	. [28 - .)	28 [14 - .)	14 [14 -17)	21 [14 - 28)
	75	. [28 - .)	. [. - .)	. [28 - .)	17 [14 - .)	28 [21 -.)

ITT- Median, 1st and 3rd quartile time-to-event with 95% CI

	Quartile	Group 1	Group 2	Group 3	Group 4	Group 5
PRNT50	25	21 [14 - 21)	14 [14 - 28)	14 [7 - 14)	14 [7 - 14)	14 [. - .)
	50	21 [21 - 28)	28 [14 - .)	14 [. - .)	14 [. - .)	14 [. - .)
	75	28 [21 - .)	. [28 - .)	17 [14 - .)	14 [14 - 28)	21 [14 - 21)

Figure 3. GMT levels over time for the groups 1-5 for PRNT90 (A) and PRNT50 (B)

Legend Figure 3. GMT levels at each visit with confidence intervals in the groups 1-5. Group 1 (3^{1}IM), group 2 ($2^{2,1}$IM), group 3 (2^{2}ID), group 4 (3^{2}ID7), group 5 (3^{2}ID14), ITT analysis



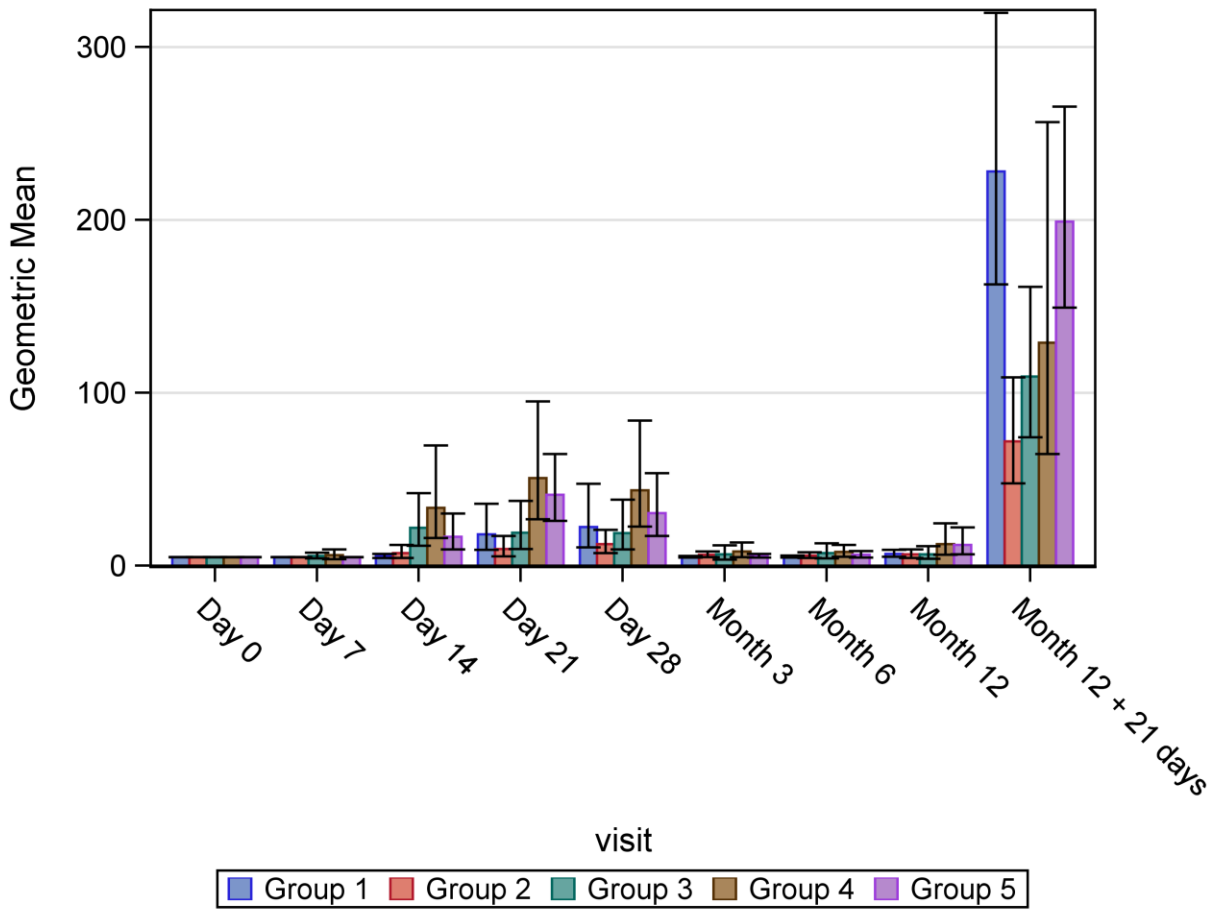


Table 1. Seropositivity (ITT analysis) in the five different groups at all visits for PRNT90 and PRNT50 (sensitivity analysis)

Seropositivity (ITT analysis) in the five different groups										
	Schedule									
	Group 1 3^1IM N = 15 n% (95% CI)		Group 2 $2^{2,1}$IM N = 16 n% (95% CI)		Group 3 2^2ID N = 15 n% (95% CI)		Group 4 3^2ID7 N = 15 n% (95% CI)		Group 5 3^2ID14 N = 16 n% (95% CI)	
	PRNT90	**PRNT50**	**PRNT90**	**PRNT50**	**PRNT90**	**PRNT50**	**PRNT90**	**PRNT50**	**PRNT90**	**PRNT50**
Day 7	0/15 0.0 (0.0 - 20.4)		0/16 0.0 (0.0 - 19.4)		1/14 7.1 (1.3 - 31.5)		1/15 6.7 (1.2 - 29.8)		0/16 0.0 (0.0 - 19.4)	
Day 14	0/15 0.0 (0.0 - 20.4)	2/15 13.3 (3.7 - 37.9)	1/15 6.7 (1.2 - 29.8)	4/15 26.7 (10.9 - 52.0)	6/15 40.0 (19.8 - 64.3)	12/15 80.0 (54.8 - 93.0)	11/14 78.6 52.4 - 92.4)	12/14 85.7 (60.1 - 96.0)	4/15 26.7 (10.9 - 52.0)	11/15 73.3 (48.0 - 89.1)
Day 21	6/15 40.0 (19.8 – 64.3)	9/15 60.0 (35.7 - 80.2)	3/16 18.8 (6.6 - 43.0)	6/16 37.5 (18.5- 61.4)	4/15 26.7 (10.9 - 52.0)	11/15 73.3 (48.0 - 89.1)	10/14 71.4 (45.4 - 88.3)	13/14 92.9 (68.5 - 98.7)	9/16 56.3 (33.2 – 76.9)	16/16 100.0 (80.6 - 100.0)
Day 28	8/15 53.3 (30.1 – 75.2)	10/15 66.7 (41.7 - 84.8)	4/16 25.0 (10.2 - 49.5)	9/16 56.3 (33.2 - 76.9)	7/15 46.7 (24.8 - 69.9)	10/15 66.7 (41.7 - 84.8)	9/13 69.2 (42.4 - 87.3)	13/13 100.0 (77.2 - 100.0)	9/16 56.3 (33.3 - 76.9)	14/16 87.5 (64.0 - 96.5)
Month 3	0/15 0.0 (0.0 - 20.4)	1/15 6.7 (1.2 - 29.8)	1/16 6.3 (1.1 - 28.3)	4/16 25.0 (10.2 - 49.5)	1/15 6.7 (1.2 - 29.8)	1/15 6.7 (1.2 - 29.8)	1/15 6.7 (1.2 - 29.8)	5/15 33.3 (15.2 - 58.3)	0/16 0.0 (0.0 - 19.4)	3/16 18.8 (6.6 - 43.0)
Month 6	0/15 0.0 (0.0 - 20.4)	1/15 6.7 (1.2 - 29.8)	1/16 6.3 (1.1 - 28.3)	2/16 12.5 (3.5 - 36.0)	2/15 13.3 (3.7 - 37.9)	3/15 20.0 (7.0- 45.2)	2/15 13.3 (3.7 - 37.9)	5/15 33.3 (15.2- 58.3)	1/15 6.7 (1.2 - 29.8)	3/15 20.0 (7.0- 45.2)

Seropositivity (ITT analysis) in the five different groups										
	Schedule									
	Group 1 3^1IM N = 15 n% (95% CI)		Group 2 $2^{2,1}$IM N = 16 n% (95% CI)		Group 3 2^2ID N = 15 n% (95% CI)		Group 4 3^2ID7 N = 15 n% (95% CI)		Group 5 3^2ID14 N = 16 n% (95% CI)	
Month 12	0/15 0.0 (0.0 - 20.4)	5/15 33.3 (15.2- 58.3)	1/15 6.7 (1.2 - 29.8)	3/15 20.0 (7.0- 45.2)	1/13 7.7 (1.4 - 33.3)	2/13 15.4 (4.3- 42.2)	5/11 45.5 (21.3 - 72.0)	6/11 54.5 (28.0- 78.7)	4/13 30.8 (12.7 - 57.6)	7/13 53.8 (29.1 - 76.8)
Month 12 + 21 days	15/15 100.0 (79.6 - 100.0)		14/15 93.3 (70.2 - 98.8)	15/15 100.0 (79.6 - 100.0)	13/13 100.0 (77.2 - 100.0)		10/11 90.9 (62.3 - 98.4)	11/11 100.0 (74.1-100.0)	13/13 100.0 (77.2 - 100.0)	

Table 2. Influence of previous yellow fever vaccination on the vaccination outcome

A.PRNT90	Yellow fever vaccination: Geometric mean ab titres, ratio, and percentages					
	Geometric mean (95% CI)		Ratio geometric means (95% CI and p-value)		n seropositive % seropositive (95% Wilson CI)	
Visit	No yellow fever vaccination	Yellow fever vaccination	Ratio (95% CI)	p-value	No yellow fever vaccination	Yellow fever vaccination
Day 0	5.00 (5.00- 5.00)	5.00 (5.00- 5.00)	1.00 (1.00- 1.00)	.	0/65 0.0 (0.0 - 5.6)	0/12 0.0 (0.0 - 24.2)
Day 7	5.29 (4.87- 5.74)	5.00 (5.00- 5.00)	1.06 (0.97- 1.15)	0.18	2/64 3.1 (0.9 - 10.7)	0/12 0.0 (0.0 - 24.2)
Day 14	8.43 (6.61- 10.7)	5.85 (4.13- 8.29)	1.44 (0.96- 2.17)	0.08	21/63 33.3 (22.9 - 45.6)	1/11 9.1 (1.6 - 37.7)
Day 21	10.4 (8.22- 13.2)	5.41 (4.53- 6.46)	1.92 (1.44- 2.55)	**<0.0001**	31/65 47.7 (36.0 - 59.6)	1/11 9.1 (1.6 - 37.7)
Day 28	10.9 (8.62- 13.8)	5.41 (4.53- 6.46)	2.02 (1.52- 2.68)	**<0.0001**	36/64 56.3 (44.1 - 67.7)	1/11 9.1 (1.6 - 37.7)
Month 3	5.57 (4.85- 6.38)	5.00 (5.00- 5.00)	1.11 (0.97- 1.28)	0.12	3/65 4.6 (1.6 - 12.7)	0/12 0.0 (0.0 - 24.2)
Month 6	5.64 (5.03- 6.33)	5.00 (5.00- 5.00)	1.13 (1.01- 1.27)	**0.04**	6/65 9.2 (4.3 - 18.7)	0/11 0.0 (0.0 - 25.9)
Month 12	6.50 (5.56- 7.61)	5.00 (5.00- 5.00)	1.30 (1.11- 1.52)	**<0.01**	11/55 20.0 (11.6 - 32.4)	0/12 0.0 (0.0 - 24.2)
Month 12 + 21 days	82.2 (66.2- 102)	43.7 (22.2- 85.9)	1.88 (1.09- 3.23)	**0.02**	55/55 100.0 (93.5-100.0)	10/12 83.3 (55.2-95.3)

B.PRNT50	Yellow fever vaccination: Geometric mean ab titres, ratio, and percentages					
	Geometric mean (95% CI)		Ratio geometric means (95% CI and p-value)		n seropositive % seropositive (95% Wilson CI)	
Visit	No yellow fever vaccination	Yellow fever vaccination	Ratio (95% CI)	p-value	No yellow fever vaccination	Yellow fever vaccination
Day 0	5.00 (5.00- 5.00)	5.00 (5.00- 5.00)	1.00 (1.00-1.00)	.	0/65 0.0 (0.0-5.6)	0/12 0.0 (0.0-24.2)
Day 7	5.40 (4.83-6.04)	5.00 (5.00-5.00)	1.08 (0.97-1.21)	0.17	2/64 3.1 (0.9-10.7)	0/12 0.0 (0.0-24.2)
Day 14	15.4 (11.5-20.7)	7.38 (3.90-14.0)	2.09 (0.99-4.41)	0.05	39/63 61.9 (49.6-72.9)	2/11 18.2 (5.1-47.7)
Day 21	28.1 (20.9-37.8)	7.26 (4.70-11.2)	3.87 (2.34-6.42)	**<0.0001**	52/65 80.0 (68.7-87.9)	3/11 27.3 (9.7-56.6)
Day 28	27.2 (20.3-36.5)	8.66 (5.09-14.7)	3.14 (1.51-6.54)	**<0.01**	52/64 81.3 (70.0-88.9)	4/11 36.4 (15.2-64.6)
Month 3	6.68 (5.58-7.98)	5.00 (5.00-5.00)	1.34 (1.12-1.60)	**<0.01**	14/65 21.5 (13.3-33.0)	0/12 0.0 (0.0-24.2)
Month 6	6.67 (5.62-7.90)	5.86 (4.62-7.44)	1.14 (0.86-1.51)	0.35	12/65 18.5 (10.9-29.6)	2/11 18.2 (5.1-47.7)
Month 12	9.43 (7.44-12.0)	5.00 (5.00-5.00)	1.89 (1.49-2.39)	**<0.0001**	23/55 41.8 (29.7-55.0)	0/12 0.0 (0.0-24.2)
Month 12 + 21 days	151 (124-183)	83.6 (43.0-163)	1.80 (1.09-2.98)	**0.02**	55/55 100.0 (93.5-100.0)	12/12 100.0 (75.8-100.0)