AV7909

STATISTICAL ANALYSIS PLAN

A Phase 2 Drug-Vaccine Interaction Study to Examine Whether Co-administering AV7909 with Ciprofloxacin or Doxycycline Affects Antibiotic Pharmacokinetics or AV7909 Immunogenicity in Healthy Adults

Clinical Protocol EBS.AVA.210

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List of Abbreviations and Definition of Terms

AE	Adverse Event
AESI	Adverse event of special interest
ANA	Antinuclear antibody
ATC	Anatomical therapeutic chemical
$AUC_{0\text{-}12h}$	Area under the serum concentration-time curve (12 hours)
$AUC_{0\text{-}inf}$	Area under the serum concentration-time curve extrapolated to infinity
$AUC_{0\text{-}t}$	Area under the concentration-time curve from time 0 to last measurable concentration
AUMC	Area under the first moment curve
AVA	Anthrax vaccine adsorbed, BioThrax
BMI	Body mass index
CBER	Center for Biologics Evaluation and Research
CI	Confidence interval
cm	Centimeter
C_{max}	Maximum observed serum concentration
CRO	Contract research organization
CSR	Clinical study report
C_t	Last measurable concentration
CV	Coefficient of variation
d	Day(s)
dsDNA	Double-stranded deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
[redacted]	[redacted]
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
e-diary	Electronic diary
ED_{50}	50% effective dilution
EWV	Early withdrawal visit
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone

GCP	Good Clinical Practice
GMT	Geometric mean titer
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
hr	Hour
ICF	Informed consent form
ICH	International Conference on Harmonisation
ID	Identification, as in subject identification (number)
IM	Intramuscular
IP	Investigational product
IRB	Institutional Review Board
ITT	Intent-to-treat
K_{el}	Apparent elimination rate constant
kg	Kilogram
LLOQ	Lower limit of quantification
LOD	Limit of detection
MedDRA	Medical Dictionary for Regulatory Activities
min	Minute
MRT	Mean residence time
NF_{50}	50% neutralization factor
NOSTASOT	No Statistical Significance Of Trend
PE	Physical examination
PI	Principal Investigator
PK	Pharmacokinetic(s)
po	Per os (orally)
q 12 hr	Every 12 hours
RF	Rheumatoid factor
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous

SD	Standard deviation
SOC	System organ class
$t_{1/2}$	Half-life
TEAE	Treatment emergent adverse event
TLF	Tables, listings, and figures
t_{max}	Time to maximum observed serum concentration
TNA	Toxin neutralizing antibody
TOST	Two one-sided t-tests
TSH	Thyroid stimulating hormone
UPT	Urine pregnancy test
US	United States
WHO	World Health Organization

1 INTRODUCTION

This Statistical Analysis Plan (SAP) is based on Protocol EBS.AVA.210 "A Phase 2 Drug-Vaccine Interaction Study to Examine Whether Co-administering AV7909 with Ciprofloxacin or Doxycycline Affects Antibiotic Pharmacokinetics or AV7909 Immunogenicity in Healthy Adults" (Version 3.0, 28 October 2019). This document specifies details of the definitions of the derived variables, analysis methods, assumptions and data handling conventions for the analyses of pharmacokinetics, immunogenicity and safety to be included in the clinical study report (CSR).

2 PROTOCOL SUMMARY

2.1. Study Objectives

2.1.1 Primary Objective

To evaluate the pharmacokinetic (PK) profiles of ciprofloxacin or doxycycline when administered orally, prior to, and following, the intramuscular (IM) administration of a two-dose schedule of AV7909 administered two weeks apart.

2.1.2 Secondary Objectives

- To assess the safety of concurrent administration of oral ciprofloxacin or doxycycline and two doses of AV7909 administered IM.
- To evaluate the Day 37 immune response using the toxin neutralizing antibody (TNA) assay following two IM doses of AV7909 with and without the concurrent oral administration of ciprofloxacin or doxycycline.

2.2 Study Design and Conduct

2.2.1 Overall Study Design

This is a randomized, open-label, phase 2, multicenter trial to investigate the potential interactions of AV7909 and ciprofloxacin or doxycycline when administered concomitantly.

Healthy males and females 18 to 45 years of age, inclusive, will read, sign and date an informed consent form (ICF) and then be screened (2 to 28 days prior to randomization) for eligibility to participate in the study. Participants (N=210) meeting the entry criteria will be evenly randomized 1:1:1 to one of the three investigational product (IP) groups shown in **Table 1**. Approximately the first 40 participants who are randomized for either IP Groups 1 and 2 will be assigned to Treatment Groups 1A or 2A while the remaining 30 participants randomized into IP Groups 1 and 2 thereafter will be assigned to Treatment Groups 1B or 2B.

Randomization will be stratified by site. A representative racial distribution will be sought among participants and sites will be asked to recruit populations that are gender- and age-balanced (i.e., between 40-60% male with 40-60% of participants in the two age ranges of 18 to 30 years of age and 31 to 45 years of age).

Table 1 Study Groups

IP Group	Treatment Group	Treatment	Sample Size (N)	
1 Cipro	1A	AV7909 + ciprofloxacin (with PK assessment)	≈40	≈70
	1B	AV7909 + ciprofloxacin (without PK assessment)	30	
2 Doxy	2A	AV7909 + doxycycline (with PK assessment)	≈40	≈70
	2B	AV7909 + doxycycline (without PK assessment)	30	
3 AV7909	3	AV7909 only	70	
		TOTAL	≈210	

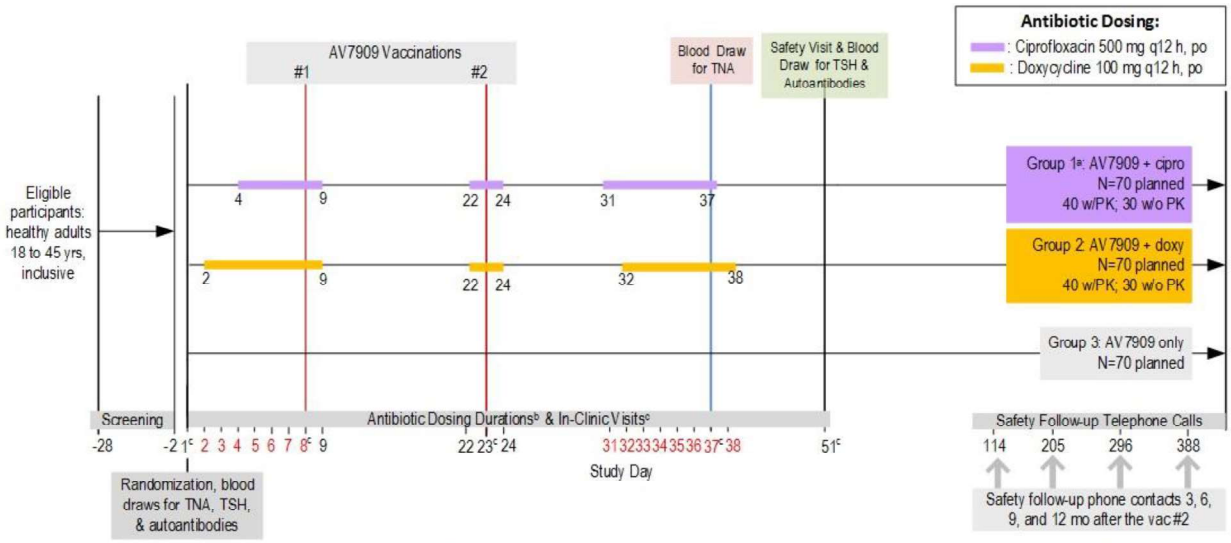
Figure 1 shows the design for this study.

Ciprofloxacin (500 mg by mouth [per os] every 12 hours [q 12 hr]) will be administered to Group 1 on Days 4-9, Days 22-24 and Days 31-37, and doxycycline (100 mg by mouth [per os] every 12 hours [po q 12 hr]) will be administered to Group 2 on Days 2-9, Days 22-24, and Days 32-38.

All participants, including the 70 participants in Group 3, will receive AV7909 (0.5 mL) IM in the deltoid muscle on Days 8 and 23. For Groups 1A and 2A, the first vaccination will occur following completion of the PK sessions and after administration of the evening antibiotic (ciprofloxacin or doxycycline) dose.

An event schematic for this study is provided in **Figure 2**.

Figure 1 Study Design for EBS.AVA.210

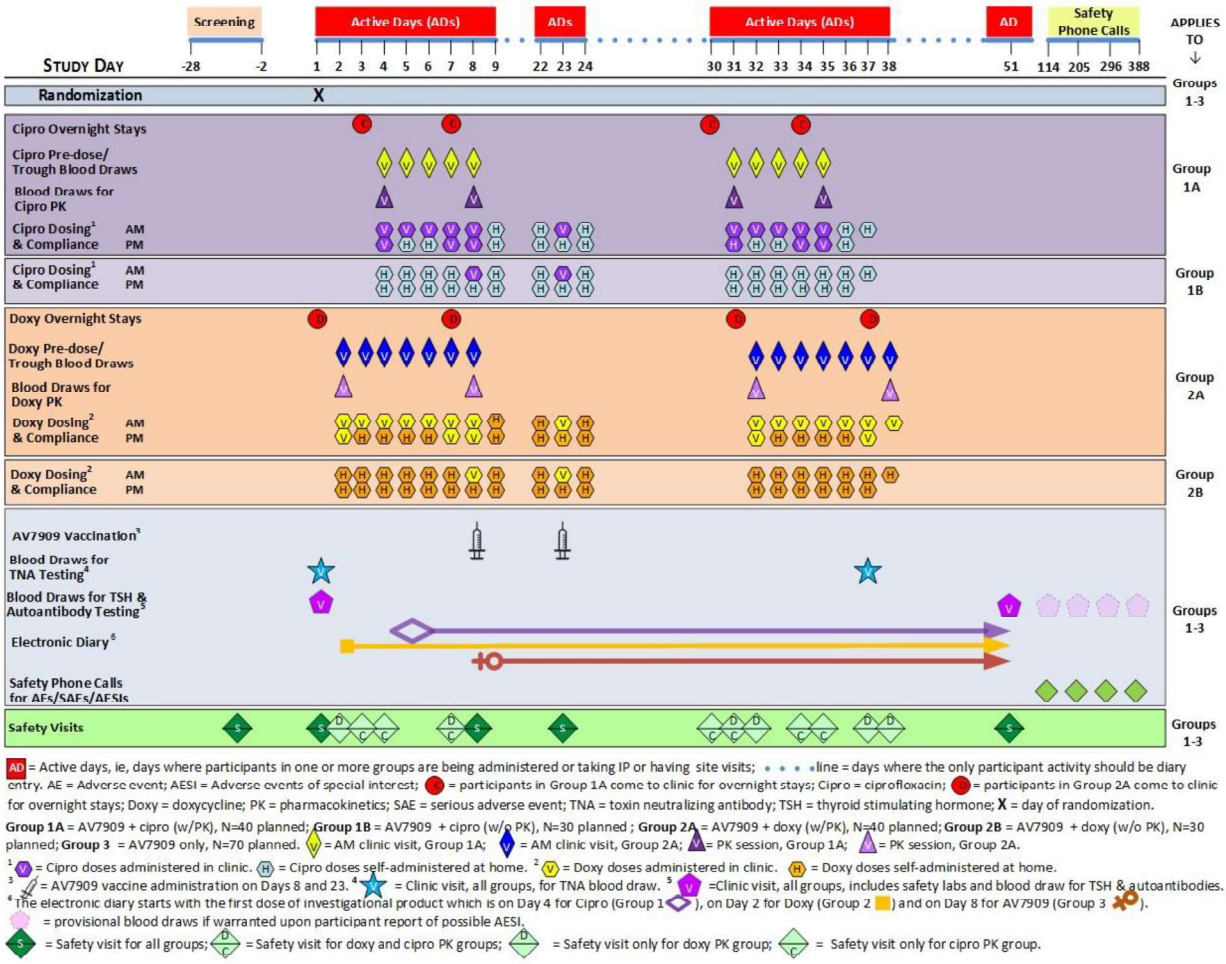


cipro = ciprofloxacin; doxy = doxycycline; PK = pharmacokinetics; po = per os, (oral); q12 h = every 12 hours; TNA = toxin-neutralizing antibody; TSH = thyroid-stimulating hormone

[a]In Group 1, the evening dose on Day 37 is not administered. [b]Antibiotic dosing for Groups 1 & 2 up to Day 8 is to enable 2 PK sessions prior to vaccination #1 (vac #1) on Day 8. Dosing continues on Day 9, extends around vac #2 (on Days 22 to 24) and then discontinues until Day 31 (cipro) or Day 32 (doxy). Cipro dosing continues from Day 31 to Day 37 and doxy dosing continues from Day 32 to Day 38, encompassing 2 PK sessions after the complete vaccination regimen as well as the blood draw for TNA. [c]Solid lines at Days 1, 8, 23, 37 and 51 are days when all groups have in-clinic visits. Days in red are those when Groups 1A and/or 2A have clinic visits for blood draws for pre-dose, trough values or PK.

All visits are performed on an outpatient basis, except Groups 1A and 2A participants will be admitted to the clinic in the evening for an overnight stay on days preceding serial (all day) blood collections needed for determination of cipro or doxy concentrations. For Group 1A, those samples will be taken on Days 4, 8, 31, and 35; for Group 2A, those samples will be taken on Day 2, 8, 32, and 38. AV7909 vac #1 will be administered to all groups on Day 8. For Groups 1B, 2B, and 3, it will be their first clinic visit after randomization and for Groups 1A and 2A, vaccination will occur in the evening following the second PK session. AV7909 vac #2 will be administered on Day 23. The final blood draw for TNA will be on Day 37 for all groups. There will be a final visit for safety for all groups on Day 51, four weeks after the last dose of AV7909, which is when the final blood draw for TSH & autoantibodies will also be collected.

Figure 2 Event Schematic for EBS.AVA.210 Groups 1 to 3



AD = Active days, ie, days where participants in one or more groups are being administered or taking IP or having site visits; • • • • line = days where the only participant activity should be diary entry. AE = Adverse event; AESI = Adverse events of special interest; = participants in Group 1A come to clinic for overnight stays; Cipro = ciprofloxacin; = participants in Group 2A come to clinic for overnight stays; Doxy = doxycycline; PK = pharmacokinetics; SAE = serious adverse event; TNA = toxin neutralizing antibody; TSH = thyroid stimulating hormone; **X** = day of randomization.

Group 1A = AV7909 + cipro (w/PK), N=40 planned; **Group 1B** = AV7909 + cipro (w/o PK), N=30 planned ; **Group 2A** = AV7909 + doxy (w/PK), N=40 planned; **Group 2B** = AV7909 + doxy (w/o PK), N=30 planned; **Group 3** = AV7909 only, N=70 planned. = AM clinic visit, Group 1A; = AM clinic visit, Group 2A; = PK session, Group 1A; = PK session, Group 2A.

[1] = Cipro doses administered in clinic. = Cipro doses self-administered at home. [2] = Doxy doses administered in clinic. = Doxy doses self-administered at home.

[3] = AV7909 vaccine administration on Days 8 and 23. [4] = Clinic visit, all groups, for TNA blood draw. [5] =Clinic visit, all groups, includes safety labs and blood draw for TSH & autoantibodies.

[6] The electronic diary starts with the first dose of investigational product which is on Day 4 for Cipro (Group 1), on Day 2 for Doxy (Group 2) and on Day 8 for AV7909 (Group 3).

= provisional blood draws if warranted upon participant report of possible AESI.

= Safety visit for all groups; = Safety visit for doxy and cipro PK groups; = Safety visit only for doxy PK group; = Safety visit only for cipro PK group.

2.2.2 PK Sampling

Serial PK assessments will be conducted on the following days:

- Group 1A (ciprofloxacin): Days 4, 8, 31, and 35;
- Group 2A (doxycycline): Days 2, 8, 32, and 38.

On these serial PK assessment days, blood samples for measurement of drug concentrations will be collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 hours post dose (± 5 min through hr 4 then ± 15 min).

In addition, antibiotic pre-dose or trough values will be measured prior to the antibiotic's morning doses on the following days:

- Group 1A (ciprofloxacin): Days 4, 5, 6, 7, and 8, and on Days 31, 32, 33, 34, and 35;
- Group 2A (doxycycline): Days 2, 3, 4, 5, 6, 7, and 8, and on Days 32, 33, 34, 35, 36, 37, and 38.

2.2.3 Immunogenicity Assessment

For all participants, blood draws for TNA assessments will be done at randomization (Day 1) and on Day 37 (± 1 day) (2 weeks following the final dose of a two-dose vaccination series). All immunogenicity laboratory samples will be evaluated using a validated TNA assay by Battelle Memorial Institute (Columbus, Ohio). Specific procedures related to collection, processing storage, and shipment of the samples will be provided in study-specific Laboratory Manual.

2.2.4 Safety Assessments

2.2.4.1 Adverse Events

AEs (including SAEs and potential AESIs) will be recorded on the AE eCRF if they occurred from the time of the first administration of any study treatment (Day 2 for Group 2, Day 4 for Groups 1 or Day 8 for Group 3) through Day 51 or EWV. From the signing of the ICF until immediately before the administration of their first study treatment, only AEs resulting from a study-related procedure will be recorded on the AE eCRF. After Day 51 and through the 12-month safety follow-up, AEs, SAEs and AEs assessed by the DSMB to be of autoimmune etiology, i.e., AESIs, will be recorded on the AE eCRF.

Safety follow-up phone contacts to collect information on AEs, SAEs and AESIs will occur at 3 months (Day 114 ± 14 days), 6 months (Day 205 ± 14 days), 9 months (Day 296 ± 14 days), and 12 months (Day 388 ± 14 days) after the last scheduled vaccination.

Refer to Protocol EBS.AVA.210, **Section 9.2** for reporting adverse events.

2.2.4.2 Clinical Laboratory Tests

All analytes to be tested during screening/safety/autoimmunity clinical laboratory tests are specified in Protocol EBS.AVA.210, **Table 10**.

The following assessments will be performed at Screening only:

- Urine drug screen
- Serologic testing (HIV-1/HIV-2 antibodies, HBV surface antigen, and HCV antibody).
- Follicle-stimulating hormone (FSH) test in postmenopausal women.

Pregnancy testing will be performed at Screening, immediately following group assignment, before each vaccination, and at the Day 51 Final Study Visit or the EWV.

Blood and urine samples for safety clinical laboratory testing (hematology, serum chemistry, urinalysis) will be collected at Screening and Day 51 as well as at the EWV if the EWV occurs before Day 51.

Blood samples for TSH & autoantibody assessment of ANA, RF and anti-dsDNA will be taken at Day 1 after randomization, Day 51 or EWV or an unscheduled visit if the participant has received at least one dose of vaccine, and at the time of safety follow-up phone contact(s) if warranted from participant report(s) of a potential AESI. These samples will be shipped to the central laboratory and will be banked; testing for TSH and ANA, RF and anti-dsDNA autoantibodies will be performed only if medically indicated (as per PI's discretion).

All samples for testing will be sent to the central laboratory for analysis, except urine for pregnancy testing, which will be performed at the site and results documented in the eCRF.

Refer to Protocol EBS.AVA.210, **Section 8.3.2** for details on clinical laboratory tests.

2.2.4.3 Physical Examination (PE)

Complete PEs will be performed at Screening, the Final Study Visit (Day 51) and EWV, if applicable.

Symptom-directed PEs will be performed at all visits except the Screening and Final Study Visit and early withdrawal (including any unscheduled visits), only if required (ie, in response to the report/collection of AEs/SAEs/potential AESIs.)

Refer to Protocol EBS.AVA.210, **Section 8.3.3** for details on physical exams.

2.2.4.4 Vital Signs

Vital signs including seated/semi-recumbent systolic and diastolic blood pressure, heart rate, respiration rate, and temperature will be obtained at Screening and each subsequent clinic visit through Day 51/EWV(except at AM and PM clinic visits for groups 1A and 2A, and at Day 37 for all groups), including unscheduled visits. Height and weight will only be recorded at Screening. On vaccination days (Days 8 and 23), vital signs will be assessed prior to vaccination and at 30 ± 5 minutes post-vaccination.

Refer to Protocol EBS.AVA.210, **Section 8.3.4** for details on vital signs.

2.2.4.5 E-diary

Participants will be asked to fill out an electronic diary (e-diary) daily for postvaccination reactogenicity events (all groups) and an e-diary for antibiotic compliance (Groups 1 and 2). There are separate e-diary requirements for this study depending on the treatment group:

- Participants in all three groups will be required to fill out e-diary to capture reactogenicity events after each vaccination.
- Participants in Group 1 and Group 2 will be required to also fill out a separate e-diary to capture antibiotic compliance.

The e-diary to capture reactogenicity events post vaccination will collect the following information:

- each participant's daily oral temperature at the same time each day
- local injection site and systemic reactogenicity responses
- whether they have use of any medications (e.g., acetaminophen, aspirin, ibuprofen)

Refer to Protocol EBS.AVA.210, **Section 8.3.5.1** for details on the e-diary and severity grading of reactogenicity responses.

2.2.4.6 Concomitant Medications

Prior and concomitant medication information (medications used 30 days prior to Screening or medications used since the last study visit) will be recorded at screening, randomization and at each study visit in the eCRF. Participants will also record concomitant medications taken in their e-diaries and site staff will review this information on a routine basis and with the participant at each study visit, transferring information to the concomitant medications eCRF page as appropriate intervals.

For the purposes of this trial, concomitant medications include prescription drugs and biologics, over-the-counter drugs as well as herbal and nutritional supplements.

2.3 Study Endpoints and Statistical Hypotheses

2.3.1 Primary Endpoints

The two primary endpoints in this study, whether co-administration of AV7909 with antibiotics affects antibiotic PK profiles for ciprofloxacin or doxycycline, are evaluated separately with independent study subjects (Group 1A for ciprofloxacin, Group 2A for doxycycline).

For each of the primary endpoints, the equivalence tests on PK parameters ($AUC_{0\text{-}12h}$ and C_{max}) at steady state pre- and post-vaccination will be performed.

Specifically, for each antibiotic, the null hypotheses are:

- $AUC_{0\text{-}12h}$: The geometric mean of the within-participant ratios of post-vaccination (Day 35 for ciprofloxacin and Day 38 for doxycycline) over the pre-vaccination (Day 8) $AUC_{0\text{-}12h}$ is greater than 1.25 or less than 0.80.
- C_{max}: The geometric mean of the within-participant ratios of post-vaccination (Day 35 for ciprofloxacin and Day 38 for doxycycline) over the pre-vaccination (Day 8) C_{max} is greater than 1.25 or less than 0.80.

The null hypothesis will be rejected when the 90% CIs of the geometric mean of the within-participant ratios is contained entirely within the equivalence bounds of [0.80, 1.25]. For each

antibiotic, to conclude that administration of two-dose regimen of AV7909 does not affect the steady-state PK profile, the null hypotheses should be rejected for both PK parameters $AUC_{0\text{-}12h}$ and C_{max}.

2.3.2 Secondary Endpoints

2.3.2.1 Secondary Safety Endpoints

- Incidences of AEs from the first dose of any IP through the Final Study Visit (Day 51)
- Incidences of serious AEs (SAEs) from the first dose of any IP until the 12-month follow-up (Day 388)
- Incidences of solicited systemic and injection site reactions reported in participant e-diaries following each vaccination
- Incidences of AESIs from the first dose of any IP until the 12-month follow-up (Day 388)
- Incidences of clinical laboratory abnormalities

No hypothesis testing is planned for any of the safety endpoints.

2.3.2.2 Secondary Pharmacokinetic Endpoints

For each antibiotic, to determine if the single-dose PK parameters are affected by AV7909 vaccination, the equivalence tests will be performed in the similar manner as those performed for the primary PK analyses.

Specifically, for each antibiotic, the null hypotheses are:

- $AUC_{0\text{-}12h}$: Geometric mean of within-participant ratios of post-vaccination (Day 31 for ciprofloxacin and Day 32 for doxycycline) over the pre-vaccination (Day 4 for ciprofloxacin and Day 2 for doxycycline) $AUC_{0\text{-}12h}$ is greater than 1.25 or less than 0.80.
- C_{max}: Geometric mean of within-participant ratios of post-vaccination (Day 31 for ciprofloxacin and Day 32 for doxycycline) over the pre-vaccination (Day 4 for ciprofloxacin and Day 2 for doxycycline) C_{max} is greater than 1.25 or less than 0.80.

No hypothesis testing is planned for the secondary PK endpoints, which will be evaluated and reported for information only.

2.3.2.3 Secondary Immunogenicity Endpoints

For each antibiotic, to evaluate whether the co-administration of the antibiotic affects the immune response after two IM doses of AV7909, a non-inferiority test will be conducted. Specifically, the null hypotheses are:

- For ciprofloxacin: the geometric mean ratio of TNA NF_{50} values at Day 37 for Group 1 over Group 3 is less than or equal to 0.5

- For doxycycline: the geometric mean ratio of TNA NF_{50} values at Day 37 for Group 2 over Group 3 is less than or equal to 0.5

The null hypothesis is rejected when the lower bound of the two-sided 95% CI of the geometric mean ratio of TNA NF_{50} values is greater than 0.5.

For each antibiotic, the formal hypothesis testing for the secondary immunogenicity endpoints will only be carried out after the primary PK endpoint has been met for the corresponding antibiotic. If the primary PK endpoint for an antibiotic is not met, the corresponding secondary immunogenicity endpoints will be provided for information only.

2.4 Sample Size and Power Considerations

The sample size for this study was selected based on both the primary and secondary endpoints.

Assessment as to whether vaccination with AV7909 affects the PK of either ciprofloxacin or doxycycline will be made based on the geometric mean of the within-participant ratios (post-vaccination over pre-vaccination) of $AUC_{0\text{-}12h}$ and C_{max}. The equivalence margin for the geometric mean ratio is [0.80, 1.25], which will be compared with the 90% confidence interval (CI) for the geometric mean of with-participant ratios of $AUC_{0\text{-}12h}$ and C_{max}. Assuming the coefficient of variation (CV) is 30% for the geometric mean ratio and the true geometric mean ratio is 0.95, 27 participants allow for 90% power to reject the null hypothesis at a significance level of 5%. A group size of 40 participants for Group 1A (AV7909 + ciprofloxacin with PK) or Group 2A (AV7909 + doxycycline with PK) is planned to allow for up to 30% of participants being excluded from the PK Population.

A sample size of 53 in each of the IP groups (Group 1 = AV7909 + ciprofloxacin, Group 2 = AV7909 + doxycycline, and Group 3 = AV7909 only) provides 90% power at 2.5% one-sided significance level for the non-inferiority test, defined as the lower bound of the two-sided 95% CI of the geometric mean ratio of the AV7909 + antibiotic over AV7909 only (Group 1 vs. Group 3, Group 2 vs. Group 3) greater than 0.5. This assumes that the true ratio of the geometric means between two groups is 0.85 and the CV of TNA NF_{50} values in each group is 100%. A total of 70 per IP group is planned to allow for up to 25% of drop-out rate.

2.5 Randomization and Blinding

2.5.1 Randomization

At the Randomization visit (Day 1), after the PI has confirmed that the participant meets all the inclusion criteria and none of the exclusion criteria, a central randomization process will be used to assign each participant to one of the three open-label IP groups with the ratio of 1:1:1 (block size of 3). Approximately the first 40 participants who are randomized into IP Groups 1 and 2 will be assigned to Treatment Groups 1A or 2A while the remaining 30 participants randomized into IP Groups 1 and 2 thereafter will be assigned to Treatment Groups 1B or 2B.

Randomization will be stratified by site. The sites will be asked to the extent possible to execute a recruitment process that achieves a representative racial/ethnic distribution in populations that are reasonably gender- and age-balanced (i.e., at least 40% male with 40% of participants in the two age ranges of 18 to 30 years of age and 31 to 45 years of age).

A randomization plan will be prepared and finalized prior to randomization of the first participant. The specific instructions for randomizing participants will be provided in the Pharmacy Manual.

A screen failure is a participant from whom informed consent is obtained and documented in writing, but who is not subsequently randomized to study treatment. Reasons for screen failure are to be recorded on the electronic case report forms (eCRFs).

Participants who are screen failures are permitted to be rescreened one time (only) according to the PI's discretion. If a participant is rescreened, a new subject ID will be assigned. To link records to the same participant, the participant's previous subject ID will be recorded in the eCRF along with the new subject ID. Participants who complete the rescreening and are randomized in the study will not be considered as screen failures.

2.5.2 Blinding

Not applicable; this is an open-label study.

3 DATA CONSIDERATIONS

3.1 Protocol Deviations

A deviation occurs when site personnel or a participant does not adhere to the protocol's stipulated requirements, whether inadvertently or planned. All identified protocol deviations will be documented (entered in the CRO's Clinical Trial Management System or equivalent), classified, and reviewed on an ongoing basis through out the study according to procedures outlined in the Protocol Deviations Management Plan (PDMP). The final protocol deviation data will be reviewed and locked prior to database lock and incorporated into Study Data Tabulation Model (SDTM).

3.2 Analysis Populations

There will be four analysis populations for this study:

The Intent-to-treat (ITT) Population will include all participants who are randomized. Participant disposition and baseline demographics will be summarized by IP group (Group 1, 2, and 3) and overall for the ITT population according to the group which the participant is randomized to.

The Safety Population will include all randomized participants who receive at least one dose of either antibiotic or AV7909. Participants will be included in the IP group (Group 1, 2, and 3) according to the treatment they received.

The PK Population will include all participants in Group 1A and Group 2A who:

- Received two doses of AV7909 according to the protocol (e.g., correct dose, no temperature excursion, and within the study-specified windows).
- If in Group 1A, received at least 5 of the 7 in-clinic ciprofloxacin doses between Day 4 through the morning of Day 8 and between Day 31 through the morning of Day

35; or if in Group 2A, received at least 7 of the 9 in-clinic doxycycline doses between Day 2 through the morning of Day 8 and between Day 32 through the morning of Day 38.

- Have adequate data for calculation of the PK parameters ($AUC_{0\text{-}12h}$ and C_{max}) at the Day 8 (both Group 1A and 2A) and Day 35 (Group 1A) or Day 38 (Group 2A) visits.
- Have no protocol deviations/events that would affect ciprofloxacin or doxycycline steady state PK assessment or immunogenicity results.

Exclusion of participants from population will be done on a case-by-case basis at the time of the PK parameter analysis.

The PK Population will be used for all PK analyses. Participants will be included in the treatment group (Group 1A or Group 2A) according to the antibiotic received.

The Immunogenicity Population will include all randomized participants who:

- Received two doses of AV7909 according to the protocol (e.g., correct dose, no temperature excursion, and within the study-specified windows).
- Had a valid immunogenicity (TNA) result at Day 1 (pre-vaccination) with no evidence of previous exposure to anthrax or an anthrax vaccine (i.e., TNA below the limit of detection of 0.059).
- Had a valid immunogenicity (TNA) result at Day 37, within the study-specified window.
- Participants in Group 1 or Group 2 should have taken at least 50% of the protocol-specified antibiotic doses.

The Immunogenicity Population will be used for the immunogenicity analyses. Participants will be included in the IP group (Group 1, 2, and 3) according to the treatment they received.

3.3 Multicenter Study

This study is expected to enroll 210 participants at approximately 4 sites in the United States. Subject randomization and protocol deviations will be summarized by site and treatment group.

3.4 Analysis Time Points

Participant visits occur on protocol-specified days with associated visit windows. The schedule may shift by ± 1 day at the following visits:

- Day 23 visit of the second vaccination for all participants.
- Day 37 visit of TNA assessment for Group 1, 2B, and 3. There is no shifting flexibility for Group 2A at this visit as PK assessment schedule at Day 38.
- Day 51 of final study visit for all participants.

If not otherwise specified, for the purposes of analysis, the analysis visits will be assigned as provided in **Table 2** for vaccination, immunogenicity (TNA) testing, clinical laboratory data,

vital signs, and physical examinations. Data at unscheduled visits will not be presented in the by-visit summary analyses but will be included in the summary tables by maximum toxicity grade or abnormality when applicable, and in data listings.

Table 2 Analysis Windows for Vaccination, Immunogenicity (TNA) Testing, Clinical Laboratory Data, Vital Signs, and Physical Examinations

Analysis Visit	Nominal Day	Lower Limit of Study Day	Upper Limit of Study Day
Baseline	NA	-28	Prior to the first exposure to any study treatment
Day 1	1	1	1
Day 8	8	8	8
Day 23	23	22	24
Day 37	37	36 for Group 1, 2B, and 3, 37 for Group 2A	38 for Group 1, 2B, and 3, 37 for Group 2A
Day 51	51	50	52

3.5 Definition of Baseline

For all analyses, the baseline value is defined as the last non-missing value prior to the first dose of any study treatment.

3.6 Selection of Data in the Event of Multiple Records in an Analysis Window

For clinical laboratory data, vital signs, and physical examinations, if multiple valid non-missing observations exist in an analysis window for a specific visit, a single value will be chosen in the by-visit summary analyses based on the following rules:

- For baseline, the last available record prior to the date and time of the first vaccination will be selected.
- For post baseline visits,
  - if the analysis values are numeric and the toxicity grades are available, the record with the highest toxicity grade will be selected;
  - if the analysis values are numeric and the toxicity grades are identical or not available, the average (arithmetic mean) will be used;
  - if the analysis values are categorical, the most conservative value will be selected (e.g., abnormal will be selected over normal).

3.7 Coding Dictionaries

Medical history and AEs will be coded to system organ class and preferred terms using Medical Dictionary for Regulatory Activities (MedDRA) dictionary version 22.0.

Medications will be coded according to the latest version of the World Health Organization's (WHO) WHODrug Global Dictionary version prior to the database lock.

3.8 Toxicity and Severity Grading Scales

The toxicity grading scales (Grade 1 to 4) that will be used for safety assessment in this study are presented in Protocol EBS.AVA.210, Appendix A (**Table 12** and **Table 13**), based on the FDA Guidance for Industry: *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials* (**CBER, 2007**). The severity of AEs, vital signs, and reactogenicity events will be assessed by the PI or the designee. Selected clinical laboratory results will be graded by the central laboratory for analytes appearing in Protocol EBS.AVA.210, Table 13 and reviewed by the PI or the designee. The toxicity grading scale has specific grading options for some clinical symptoms, such as nausea/vomiting, diarrhea, headache, fatigue, and myalgia. For symptoms not appearing on the grading scale, the grading for generic "illness or clinical AE" will be used (refer to Protocol EBS.AVA.210, Table 12). For reactogenicity assessment, a Grade of 0 will be available in the e-diary to record "symptom not present".

4 STATISTICAL ANALYSIS

4.1 General Considerations

Data summaries will be tabulated by appropriate grouping for each analysis population as specified in **Section 3.2**. Continuous variables will be summarized by descriptive statistics including number of non-missing observations (n), mean, standard deviation (SD), median, minimum and maximum. Categorical variables will be summarized by the frequency count (n) and the percentage of participants (%) in each category. Logarithm transformation will be used when appropriate.

All clinical laboratory data and vital sign data will be reported using standard international units, if applicable. The immunogenicity response TNA NF_{50} is a unitless quantitative result as it is derived from ED_{50} divided by the reference standard of ED_{50}.

The reporting conventions in **Appendix I** will be applied to all tables, listings, and figures. All derivations, statistical analyses, summaries, and listings will be generated using SAS Version 9.4 or higher (SAS Institute, Inc., Cary, North Carolina, United States).

4.2 Precision

Safety variables (i.e., clinical laboratory values, vital signs) including derivations thereof will be reported to the same precision as the source data. The immunogenicity response TNA NF_{50} will be reported with one decimal place or two significant digits (e.g., 0.032, 18.0).

All PK concentrations will be reported and analyzed with the same precision as the source data provided by the bio-analytical laboratory regardless of how many significant figures or decimals the data carry. Derived PK parameters will be rounded for reporting purposes in by-subject listings. The unrounded derived PK data will be considered the source data for the calculation of descriptive statistics and the statistical analysis. For most derived PK parameters, 3 significant digits will be used as the standard rounding procedure, with the following exceptions:

- Parameters directly derived from source data (e.g., C_{max}) will be reported and analyzed with the same precision as the source data.
- Parameters derived from actual elapsed sample collection times (e.g., t_{max}) will be reported with the same precision as the actual elapsed sampling time value of the source data. Actual elapsed time from dosing will be rounded to 2 decimal places.

Coefficient of variation will be reported to 1 decimal place. Ratios of means for pharmacokinetic parameters will be presented with two decimal places.

4.3 Derived Variables

In all data listings, study day(s) relative to Study Day 1 (date of randomization) will be presented. The day prior to the date of randomization is Study Day -1. There is no Study Day 0.

Study day relative to Study Day 1 will be calculated as:

- Study day = (assessment date – date of randomization) if the assessment is before the date of randomization.
- Study day = (assessment date – date of randomization + 1) if the assessment is on or after the date of randomization.

If the study day is missing due to missing dates, it will remain as missing unless specified otherwise.

4.4 Handling of Missing Data

Descriptive safety data summary will be based on observed cases. For imputation of completely and partially missing dates of AEs or prior and concomitant medications, if appropriate, the following conventions will be used:

- For the start date missing completely, impute the date of the first exposure to any study treatment.
- For the start date with missing day only, impute the 1st of the month unless month is same as month of the first exposure to any study treatment then impute the date of first exposure to any study treatment.
- For the start date with missing month and day, impute 1st January unless year is the same as the date of the first exposure to any study treatment then impute the date of first exposure to any study treatment.
- If the stop date is not missing and the start date is after the stop date after missing value calculation, the stop date will be used for the start date.
- For the stop date missing completely, impute date of last contact.
- For the stop date with missing day only, impute the last day of the month. If the month of the stop date is same as month of last contact then impute the date of last contact.
- For the stop date with missing month and day, impute 31st December or date of last contact, whichever is earlier.

- An AE completely missing both start and stop dates, or with the start date missing and stop date later than the first dose date of study drug, will be considered to be treatment- emergent.

Missing start/stop dates will remain as missing in the participant data listings but will be imputed to permit proper tabulation of AE and concomitant medications data.

Missing PK concentrations and missing PK sampling time will be handled as described in **Section 6.3** and **6.4**.

Unless specified above, no other imputation will be made for missing data.

4.5 Diary Data Issue Handling

4.5.1 Oral Temperature Data Errors

Oral temperatures are reported by participants in the e-diary for post vaccination reactogenicity. The numeric temperature values and the associated toxicity grades with temperatures less than 90 °F or greater than 110 °F will be set to null in the analysis.

4.5.2 Antibiotic Dosing Data Errors

Antibiotic self-administered at home will be reported by participants in the antibiotic e-diary. Issues related to entry of information into antibiotic e-diary will be manually reviewed by monitors and resolutions will be performed on a case-by-case basis.

4.6 Adjustment for Covariates

An exploratory immunogenicity linear regression analysis will be conducted with adjustment for site, and covariates age and sex, as specified in **Section 7.2.1**.

4.7 Subgroup Analysis

For immunogenicity analysis, TNA NF_{50} values will be summarized by treatment group and each of the following classification variables (categories are defined in the parentheses):

- Age (18 - 30, 31 - 45)
- Sex (Male, Female)

No statistical testing will be performed for subgroup analyses.

4.8 Multiplicity Adjustment

There is a single primary endpoint for each of the two antibiotics, which will be evaluated separately with independent study participants (Group 1 for ciprofloxacin, Group 2 for doxycycline). Demonstration of non- interference of steady state PK is defined separately and there is only one way to meet the primary endpoint for each antibiotic. Success is therefore defined per antibiotic only, not at the study level, and type I error is controlled for each

antibiotic. As such, multiplicity adjustment is not needed between the endpoints across the two antibiotics.

For the assessment of the steady state PK as the primary endpoint, there are two parameters, $AUC_{0\text{-}12h}$ and $C_{max.}$ To conclude that administration of two-dose regimen of AV7909 does not affect the steady state PK of each antibiotic, the null hypotheses should be rejected for both parameters of the corresponding antibiotic. For each parameter, the type I error rate is controlled at 5% and there is no need to adjust for multiple testing when the criteria for both parameters must be met.

Of the secondary endpoints for each antibiotic, formal hypothesis testing will only be carried out for the immunogenicity endpoints and only after the primary PK endpoint has been met for the corresponding antibiotic. If the primary PK endpoint for an antibiotic is not met, the corresponding secondary immunogenicity endpoint will be provided for information only. Non-inferiority of the secondary immunogenicity endpoint is evaluated with a one-sided alpha of 0.025. This pre-specified hierarchical order of hypotheses testing preserves the type I error rate without the need for further adjustment.

No other formal hypothesis testing will be carried out. The secondary PK endpoints (single dose antibiotic PK values) and all safety endpoints will be evaluated and reported for information only. Thus no multiplicity adjustment is needed.

5 STUDY POPULATION CHARACTERISTICS

5.1 Subject Disposition

Subject disposition will be displayed in form of tabulation and listing for all participants who signed and dated the ICF. Tabulations will include the number of participants screened, randomized, treated with any study treatment, completed the study, discontinued study treatment, and study withdrawal. Reasons for discontinuation of treatment and study withdrawal will be summarized.

The participant last study visit will be summarized by treatment group and overall for all randomized participants. The number and percentage of participants randomized by site will be provided by treatment group and overall based on the ITT population. Reasons for screen failure and reasons for exclusion from PK Population and Immunogenicity Population will be summarized.

Listings of participants excluded from the PK Population and Immunogenicity Population with reasons will be provided.

5.2 Protocol Deviations

Protocol deviations defined in **Section 3.1** will be summarized by deviation category, severity, and treatment group for each site and overall based on the ITT Population.

A listing of protocol deviations will be provided.

5.3 Demographics and Baseline Characteristics

5.3.1 Demographics

The following demographics and baseline characteristics will be summarized as continuous variables using descriptive statistics (n, mean, SD, median, minimum, and maximum) by IP/treatment group and overall for all participants in the Safety, ITT, PK, and Immunogenicity populations:

- Age (years)
- Baseline weight (kg)
- Baseline height (cm)
- Baseline BMI

The following demographics and baseline characteristics will be summarized as categorical variables with counts and percentages by IP/treatment group and overall for ITT, Safety, PK, and Immunogenicity populations:

- Age (18 – 30, 31 – 45 years)
- Sex (Female, Male)
- Race (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, More than one Race, and Other)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino, Unknown)

Subject demographics and baseline characteristics data listing will be provided.

5.3.2 Medical History

Medical history will be coded to system organ class (SOC) and preferred term (PT), according to the Medical Dictionary for Regulatory Activities (MedDRA). A summary table and a listing of medical history will be provided.

5.4 Treatment Compliance and Exposure to Study Drugs

5.4.1 Compliance of Ciprofloxacin and Doxycycline

The compliance rate of ciprofloxacin and doxycycline will be computed for each participant who takes at least one dose of antibiotic as 100 times the total number of tablets taken (administered in-clinic and reported in the CRF by staff and self-administered by participants at home and reported to the e-diary) divided by the required total number of tablets that should have been taken as specified in **Figure 2**. The compliance will be summarized as follows by appropriate treatment group:

- Number of participants taking at least 50% of protocol specified doses

- Number of participants in Group 1A taking at least 5 of 7 in-clinic ciprofloxacin doses or in Group 2A taking at least 7 of 9 in-clinic doxycycline doses prior to each PK assessment.

The subject listing of antibiotic administration will be provided.

5.4.2 AV7909 Compliance and Exposure

AV7909 will be administered to all participants in all IP groups as a 0.5 mL IM vaccination in alternate arms on Days 8 and 23. Vaccinations will be administered in the clinic by authorized personnel in a controlled, clinical environment.

AV7909 exposure will be tabulated as the number and percentage of participants receiving each vaccination by treatment group for all vaccinations administered and for per-protocol vaccinations which are administered without any administration issues, e.g., incorrect product administered, incorrect dose of IP, incorrect route, missing previous dose, and outside the acceptable visit windows on Days 8 and 23(± 1 day). Shift of the first vaccination at Day 8 in Group 3 may be acceptable with consideration of subject overall vaccination and assessment schedules.

The subject listing of AV7909 vaccinations will be provided.

6 PK ASSESSSMENT

6.1 Serum Concentration Data

Subjects with partial data will be evaluated on a case-by-case basis to determine if sufficient data are available for reliable estimation of PK parameters.

A listing of PK blood sample collection times as well as derived sampling time deviations will be provided.

6.2 Exclusion of Outliers

Individual serum concentrations may be excluded from the analysis because they are erroneous or abnormal at the discretion of the pharmacokineticist following a review of available documentation (e.g., bioanalytical report, validation report) and communication with the sponsor. Any such exclusion will be clearly listed in the study report along with justification for exclusion.

Entire serum concentration-time profiles for a participant may be excluded following review of available documentation (e.g., bioanalytical report, validation report, and protocol deviation log) and communication with the sponsor. However, results of analysis with and without the excluded profiles may be presented in the study report. Any such exclusion will be clearly listed in the study report along with justification for exclusion.

Any excluded data will be flagged in the individual data listings.

6.3 Non-Quantifiable and Missing Concentrations

If a concentration value is below the lower limit of quantification (<LLOQ), which is 10 ng/mL for the ciprofloxacin assay and 100 ng/mL for the doxycycline assay:

- Individual concentration listings in the CSR should list these concentrations as "<LLOQ".
- For the calculation of concentration summaries and plotting mean and individual concentration-time profiles, concentration values <LLOQ are treated as "zero".

For purpose of calculating PK parameters, pre-dose concentrations <LLOQ, missing pre-dose values (Day 4 Group 1A and Day 2 Group 2A, only), and concentrations prior to the first quantifiable concentration that are <LLOQ are set to "zero". A value <LLOQ occurring after C_{max}, that is embedded between two quantifiable points will be treated as missing for PK parameter calculation. Values <LLOQ occurring at the end of the collection interval (after the last quantifiable concentration value) will be set to "zero" to allow for calculation of $AUC_{0\text{-}12h}$. For calculation of $AUC_{0\text{-}inf}$, trailing zero values are treated as missing by Phoenix WinNonlin. If there are more than two consecutive <LLOQ concentrations after C_{max}, all concentrations after that may be treated as missing after pharmacokineticist review of available documentation (e.g., bioanalytical report, validation report).

For Handling of missing data for $AUC_{0\text{-}inf}$ and $AUC_{0\text{-}12h}$ calculation is described below in Section 6.4.

6.4 PK Parameters

Where possible, the PK parameters in **Table 3** for ciprofloxacin and doxycycline will be derived from the concentration-time data by non-compartmental techniques using actual elapsed time from dosing. If the actual PK sampling time is missing, but a valid concentration value has been measured, the scheduled protocol time may be used for the calculation of derived PK parameters.

Any anomalous concentration values observed at predose for single dose PK days will be identified in the clinical study report and used for the computation of PK parameters. Pharmacokinetic parameters will be computed irrespective to whether the anomalous concentration exceeds 5% of the observed maximum concentration (C_{max}). If the anomalous predose concentration is greater than 5% of C_{max}, the PK parameters for the affected subject profile will be calculated and reported in the listing but excluded from statistical summaries and analyses. Similarly, PK concentrations for the affected profile will be excluded from statistical summaries.

Non-compartmental PK parameter calculations will be performed using Phoenix® WinNonlin® 8.0 or higher (Certara Company, Princeton, New Jersey, United States) or SAS version 9.4 or higher. Graphics may be prepared using the same versions of SAS, or Phoenix WinNonlin, or with SigmaPlot 12.5, or higher (Systat Software, Inc., San Jose, California, United States).

Table 3 Ciprofloxacin and Doxycycline PK Parameters

Variable	Definition
C_{max}	Maximum observed serum concentration
t_{max}	Time of maximum observed serum concentration
$t_{1/2}$	Half-life (calculated as ln 2/ K_{el})
K_{el}	Apparent elimination rate constant; a minimum of 3 data points in the regression interval will be required for determination
AUC_{0-12h}	Area under the serum concentration-time curve (12 hours)
AUMC	Area under the first moment curve
AUC_{0-inf}	Area under the serum concentration-time curve extrapolated to infinity
MRT	Mean residence time

The linear-up/log-down trapezoidal summation will be used for calculation of AUC parameters. AUC_{0-inf} will only be calculated if K_{el} can be estimated. If K_{el} cannot be estimated due to missing data or an undetermined terminal phase, AUC_{0-inf} will not be calculated. The reliability of estimation of K_{el} and hence the PK parameters AUC_{0-inf} and $t_{1/2}$ will be also be evaluated using diagnostic parameters (adjusted Rsq, time interval of the log-linear regression to determine K_{el}, percentage of AUC_{0-inf} obtained by extrapolation). If the adjusted Rsq is <0.800, then the K_{el}-related parameters AUC_{0-inf} and $t_{1/2}$ will only be listed but not included in summary statistics. Any exclusions will be documented in the clinical study report.

AUC_{0-inf} will only be calculated for single-dose serial PK days; for steady-state, only AUC_{0-12h} (i.e., AUC_{0-tau}) will be reported.

For calculation of AUC_{0-12h}, if the t=12h sample is missing or collected outside of the protocol-specified window of ±15 min, AUC_{0-12h} will be determined to a nominal time point of 12 hours using interpolation or extrapolation (if K_{el} can be estimated; trailing BLQ values are to be set to missing in that case). For extrapolation, if K_{el} cannot be estimated due to missing data or an undetermined terminal phase, AUC_{0-12h} will not be calculated. Interpolation will not be performed if the subsequent concentration is <LLOQ and AUC_{0-12h} will not be calculated.

For multiple-dose profiles, if the concentration at time of dosing is missing then the concentration will be set to equal the concentration at the end of the dosing interval (assuming linear PK and steady-state conditions). For multiple-dose profiles with missing data at the end of the dosing interval for which interpolation/extrapolation is not possible, the missing concentration will be set to the pre-dose value to allow for calculation of AUC_{0-12h}.

6.5 PK Analyses

All PK analyses will be conducted based on the PK Population. All PK data listings will be presented based on the Safety Population.

PK concentration data will be summarized by study day and nominal time. The trough concentrations will be summarized by study day. The summary statistics for concentration data will include n, arithmetic mean, SD, median, minimum, maximum, and coefficient of variation (CV%).

PK parameters in **Table 3** will be summarized by study day. Summary of t_{max} will include n, median, minimum, and maximum only. For other PK parameters, n, arithmetic mean, SD, median, minimum, maximum, coefficient of variation (CV%), geometric mean, and geometric CV% will be presented.

The CV% is computed as:

CV% = 100x(SD/Mean).

The geometric mean is computed as:

Geometric mean = exp(arithmetic mean of log transformed data).

The geometric CV% is computed as:

CV% geometric mean = (sqrt (exp (variance for log transformed data)-1))x100.

Individual as well as arithmetic mean concentration-time profile plots for serum concentration and trough concentrations will be presented in linear and semi-logarithmic scale.

The scatter plots of individual and geometric mean values for $AUC_{0\text{-}12h}$ and C_{max} for ciprofloxacin and doxycycline will be presented for single dose and steady state profiles. The scatter plots of individual and geometric mean values for the ratio of $AUC_{0\text{-}12h}$ and C_{max} will be presented for single dose and steady state profiles.

6.5.1 Equivalence Testing for Primary PK Endpoints

For each of the two antibiotics, the goal with the primary PK endpoint is to demonstrate that vaccination with AV7909 does not affect the steady-state PK profile. The equivalence testing will be constructed using paired two one-sided t-tests (TOST) to provide the point estimate and 90% CIs for geometric mean of the within-participant ratios of the steady-state values of $AUC_{0\text{-}12h}$ and C_{max} post two doses of AV7909 (Day 35 for ciprofloxacin and Day 38 for doxycycline) vs. pre-vaccination of AV7909 (Day 8 for ciprofloxacin and doxycycline). The analyses will be conducted with natural log transformation of PK parameters. Results obtained from transformed analyses will be back-transformed by exponentiation for presentation. To conclude that AV7909 vaccine has no effect on the steady-state PK of ciprofloxacin or doxycycline, the 90% CIs should fall entirely within the equivalence range of [0.80, 1.25] for both $AUC_{0\text{-}12h}$ and C_{max}.

6.5.2 Equivalence Testing for Secondary PK Endpoints

For each antibiotic, the secondary PK analyses will be performed to evaluate the effect of vaccination with AV7909 on the single-dose PK profile. The equivalence tests will be conducted in the similar statistical manner as the primary analyses. If the 90% CIs for geometric mean of the within-participant ratios of the single-dose values of $AUC_{0\text{-}12h}$ and C_{max} post two doses of AV7909 (Day 31 for ciprofloxacin and Day 32 for doxycycline) vs. pre-vaccination of AV7909 (Day 4 for ciprofloxacin and Day 2 for doxycycline) fall entirely within the equivalence range of [0.80, 1.25] for both $AUC_{0\text{-}12h}$ and C_{max}, it is concluded that AV7909 vaccine has no effect on the single-dose PK for ciprofloxacin or doxycycline.

6.5.3 Time to Steady State Assessment Using Trough Concentrations

To evaluate time to steady state before and after AV7909 vaccination, the trough levels of ciprofloxacin will be analyzed by comparing values on Days 4, 5, 6, 7, and 8 and on Days 31, 32, 33, 34 and 35; the trough levels of doxycycline will be analyzed by comparing values on Days 2, 3, 4, 5, 6, 7, and 8 and on Days 32, 33, 34, 35, 36, 37 and 38.

Stepwise testing for linear trend, the application of the NOSTASOT (No Statistical Significance Of Trend) methodology to trough concentrations proposed by **Maganit et al., 2008** involves straight line approximations to the aggregate plasma concentration curve over specified time intervals; therefore, the null hypothesis is that there is no linear trend, i.e., the slope of the regression line equals zero. The alternative is that there is a linear trend (slope not equal to zero). The first linear contrast uses the entire range of time points included in the model. If the contrast is significantly different from zero at alpha=0.05, a new linear contrast is tested, this time excluding the earliest time point. This testing continues, based on contrasts successively excluding the next earliest time point from the start of the study, until the contrast is no longer significantly different from zero or until only three timepoints remain in the contrast. If the final contrast is not statistically significant, then the first timepoint included in that contrast is considered to be the time point at which steady state is attained. If the final contrast includes three timepoints and is still statistically significant, then steady state is considered not to have been attained by the end of the study.

The repeated measures ANOVA model will be conducted with natural log transformed trough level as response and study day as fixed factor. The slope estimates along with the 90% confidence intervals for the slope will be presented as results.

7 IMMUNOGENICITY ANALYSIS

All the immunogenicity analyses presented in the tabulations and figures will be performed based on the Immunogenicity Population as defined in **Section 3.2**. The immunogenicity data listing will be presented based on the Safety Population.

Immunogenicity is assessed with TNA NF_{50}. In the tabulations and figure, the non-missing TNA NF_{50} values which are below the LLOQ will be imputed with 0.032, which is ½ the LLOQ of the assay (**BBRC Study Report 762-G004690**). In the data listing, TNA NF_{50} values which are below the LLOQ will be presented in the original values.

7.1 Summary of Immunogenicity Data

The summary of immunogenicity response TNA NF_{50} at Day 37 and IP group (Group 1, 2, 3, and Total) will include:

- Descriptive summaries (n, mean, median, standard deviation, minimum, and maximum) of TNA NF_{50} values
- Geometric mean titer (GMT) and corresponding 95% CIs for TNA NF_{50}

Subject data listing with TNA assessments at baseline (prior to administration of ciprofloxacin or doxycycline on Day 1) and at Day 37 (2 weeks following the final dose of a two-dose vaccination series) will be presented.

The scatter plot of estimated GMTs and associated 95% CIs for TNA NF_{50} at Day 37 by treatment group will be provided. In addition, scatter plots of estimated GMTs and associated 95% CIs for TNA NF_{50} at Day 37 will be presented by each subgroup (age group and sex) in each treatment group.

7.2 Secondary Immunogenicity Endpoints

The secondary immunogenicity analysis is aimed to assess whether the immunogenicity of AV7909 two weeks following the final dose of a two-dose vaccination series is affected by concomitant dosing with oral ciprofloxacin or doxycycline.

Non-inferiority test will be constructed using the GMT ratio of TNA NF_{50} (Group 1/Group 3 or Group 2/Group 3) between IP group with participants that received both AV7909 and ciprofloxacin or doxycycline (Group 1 or 2) and the IP group with participants that received AV7909 only (Group 3). Point estimate and the 95% CI for the GMT ratio will be estimated using the linear regression based on log 10 transformed TNA NF_{50} with equal variance. If the lower bound of the two-sided 95% CI of geometric mean ratio is greater than the non-inferiority margin of 0.5, it is concluded that the immune response in the group who received AV7909 plus either ciprofloxacin or doxycycline is non-inferior to the group which received AV7909 alone and thus that either ciprofloxacin or doxycycline did not demonstrably affect the immunogenicity of AV7909.

7.2.1 Exploratory Analysis for Immunogenicity

To evaluate the possible impact of imbalance in sex and age on the immunogenicity endpoints, exploratory analyses will be conducted using linear regression to compare the immunogenicity response at Day 37 between each AV7909 + antibiotic group and the AV7909 only group, i.e., between Group 1 (AV7909 + ciprofloxacin) and Group 3 (AV7909) and between Group 2 (AV7909 + doxycycline) and Group 3 (AV7909). In the linear regression models, the stratification factor, study site, will be included in the model as a random effect; treatment group, participant sex and age will be included as fixed factors with age being dichotomized into ≤30 years and >30 years. The adjusted GMT ratio for TNA NF_{50} will be provided with 95% confidence interval. Any differences between the exploratory (adjusted) and the primary (unadjusted) analyses will be discussed in the final CSR.

8 SAFETY ANALYSIS

All safety data will be presented in the form of tabulations and listings, based on the Safety Population. Data summaries will be tabulated by treatment group as specified in **Section 3.2**.

8.1 Adverse Events

AEs will be coded to system organ class (SOC) and preferred term (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA). A subject data listing of all AEs (including treatment-emergent AEs (TEAEs) and non-TEAEs) sorted by treatment group, subject ID and AE start date/time will be provided based on the Safety Population. This listing

will include a "non-treatment-emergent" flag and the date of the last IP dose. Only TEAEs will be included in the AE summaries described below.

8.1.1 Overall Summary of TEAEs

A treatment emergent adverse event (TEAE) is defined as an AE that presents after the initiation of treatment or any AEs already present that worsen in either intensity or frequency following treatment. For programming purpose, any AEs with start date on or after the date of first exposure of any study treatment will be counted as treatment emergent. AEs with missing start dates, but with stop dates overlapping into the treatment period will also be counted as treatment emergent.

An overall summary of the following TEAE categories will be provided:

- TEAEs
- TEAEs related to any study treatment
- Grade 3/4 TEAEs
- Grade 3/4 TEAEs related to any study treatment
- Serious TEAEs
- Serious TEAEs related to any study treatment
- TEAEs leading to death
- TEAEs leading to discontinuation of any study treatment
- TEAEs leading to study withdrawal
- AESIs
- AESIs related to study vaccine

8.1.2 All TEAEs

All TEAEs will be tabulated by SOC and PT (in descending order of participant incidence in "AV7909 Total" column).

TEAEs with 5% or more for a PT term in any treatment group will be tabulated by SOC and PT.

All TEAEs will be summarized by SOC, PT, severity, and treatment group. The severity of AEs will be assessed by the PI or designee using toxicity grading (Grade 1 to 4) according to Protocol EBS.AVA.210, **Appendix A**. All TEAEs will also be summarized by SOC, PT, relationship to study treatment, and treatment group. Participants having the same TEAE more than once will be counted once for each PT and once within each SOC at the maximum severity and relatedness. If the severity/relatedness is missing for one or more of the occurrences, the maximum severity/relatedness of the remaining occurrences will be used.

TEAE summaries will be provided for TEAEs through the Final Study Visit (Day 51) and for all TEAEs through the 12-month follow up , respectively.

8.1.3 Drug/Vaccine Related TEAEs

Adverse event causal relationship to the study treatment will be assessed by the PI or designee using terms and scales "Unrelated", "Possibly related", "Probably related", and "Definitely related". If the relationship between the AE and the study treatment is determined to be "Possibly" or "Probably" or "Definitely related", the event will be considered as related to the IP.

To assess the causality between AEs and the study treatment ciprofloxacin, doxycycline, and AV7909, TEAEs will be tabulated with the structures as follows:

- TEAEs by SOC, PT, and Relationship to Study Vaccine.
- TEAEs by SOC, PT, and Relationship to Antibiotics.
- TEAEs onset prior to the first vaccination by SOC, PT, and relationship to ciprofloxacin or Doxycycline.

8.1.4 Serious TEAEs

Serious TEAEs will be tabulated using the MedDRA coded terms of SOC and PT by relationship to study treatment (ciprofloxacin, doxycycline, AV7909). A subject data listing of all serious TEAEs will be provided.

8.1.5 Adverse Events of Special Interest (AESI)

Treatment-emergent AESIs determined to be of autoimmune etiology based on decision of the DSMB will be tabulated by SOC, PT, and relationship with AV7909. A subject data listing of all AESIs will be provided.

8.1.6 TEAEs Leading to Discontinuation of Study Treatment, and Study Withdrawal

Separate tabulations will be displayed for TEAEs leading to discontinuation of antibiotic or vaccination, and withdrawal from the study.

8.1.7 Deaths

A large number of deaths is not expected in this study with healthy participants. A tabulation and subject data listing of all deaths will be provided as appropriate.

8.2 Clinical Laboratory Tests

Clinical laboratory results for the lab analytes that appear in Protocol EBS.AVA.210, **Table 13** will be assigned by the central laboratory a toxicity grade (Grade 1=mild through Grade 4=potentially life-threatening).

Central laboratory measurements will be tabulated by treatment group as follows:

- Observed values and changes from baseline of continuous laboratory variables (hematology, serum chemistry, and selected urinalysis parameters [e.g., specific

gravity]) will be summarized using descriptive statistics (n, mean, SD, median, minimum, and maximum) by study visit and treatment group.

- Observed values of categorical laboratory analytes (e.g., urinary protein) and shifts from baseline (number and percentage) will be summarized by study visit and treatment group.
- Shift from baseline according to normal reference ranges (reported as 'Low', 'Normal', and 'High') will be summarized (number and percentage) by study visit and treatment group.
- Shift from baseline according to toxicity grading criteria grade (Grade 0, Grade 1-4) will be summarized (number and percentages) by study visit for analytes that appear in Protocol EBS.AVA.210, **Table 13**. Grade 0 includes all values within normal range or value not meeting criteria for toxicity of at least Grade 1.
- Frequency and percentage of participants with the highest grade of post baseline abnormal laboratory values according to toxicity grading criteria grade (Grade 1- 4).
- Post baseline abnormal laboratory results with Grade 3 or higher

All laboratory records will be provided in the subject data listings. This listing will include all test results that are collected throughout the study for the analyte of interest, with all applicable severity grades or abnormal flags displayed.

8.3 Vital Signs

Vital sign data will be assigned a toxicity grade (Grade 1=mild through Grade 4=potentially life-threatening) according to Protocol EBS.AVA.210, **Table 12**.

Vital signs data will be summarized as follows:

- Observed values and changes from baseline by study visit and treatment group.
- Shift from baseline according to toxicity grading criteria grade (Grade 0, Grade 1-4) (number and percentages) by study visit and treatment group. Grade 0 includes all values not meeting criteria for toxicity of at least Grade 1.

All vital sign records will be provided in the subject data listings. This listing will include all results that are collected throughout the study for the analyte of interest, with all applicable severity grades displayed.

8.4 Physical Examinations

Complete and symptom-directed PE findings will be tabulated including number of participants with normal/abnormal PE findings by study visit, body system, and treatment groups. Listings will be provided.

8.5 Prior and Concomitant Medications

Prior and concomitant medication information will be coded according to the World Health Organization's (WHO) WHODrug Global Dictionary. Data will be tabulated by Anatomical

Therapeutic Chemical (ATC) classification level 4, medication preferred term, and treatment group. A subject data listing of all medications will be provided.

Prior medications are those used from within 30 days before screening through the start of the first study treatment (ciprofloxacin, doxycycline, or AV7909), while concomitant medications are those used since the start of the first IP. For purposes of analysis, any medication with a stop date between 30 days before date of screening and prior to the start date of the first study treatment will be categorized as prior; Any medication which is ongoing or with a stop date that is on or after the start date of the first study treatment will be categorized as a concomitant medication. Partial dates will be imputed according to **Section 4.4**. In the tabulation, ATC levels and standardized names within each ATC level are sorted in descending order of percentage in the Total column. A subject data listing of all medications will be provided.

8.6 Other Safety Analyses

8.6.1 E-diary Reactogenicity

E-diary reactogenicity data will be summarized with number and percentage of participants with the highest severity grade for each symptom of solicited systemic reactions (tiredness, muscle ache, headache, and fever) and injection site reactions (warmth, tenderness, itching, pain, arm motion limitation, redness, induration, swelling, and bruising) by severity grade and treatment group post each vaccination. Severity of fever will be attributed programmatically using oral temperature measurement reported in the e-diary according to the grading scale for fever in Protocol EBS.AVA.210, **Table 12**.

Participant listings of e-diary reactogenicity will be provided for each symptom of systemic and injection site reactions.

8.6.2 In-clinical Reactogenicity

In-clinical reactogenicity data will be summarized in the similar manner as those for e-diary reactogenicity, except for fever (oral temperature) which will be reported and presented as vital sign observations at 30 minutes post vaccinations.

All in-clinical reactogenicity data will be provided in the data listings.

8.6.3 E-diary Compliance

The compliance of reactogenicity e-diary will be summarized by vaccination and treatment group. Degree of compliance for a participant and vaccination will be calculated based on the expected total number of diary days, including extra days if there was an ongoing reaction, times the number of questions per day. The subject-level compliance percentage will be calculated for each vaccination, and then summarized with the following categories: Did not enter any diary, < 50%, 50-75%, and >75%.

Participants in Groups 1 and 2 are required to complete antibiotic e-diary entries to record the time of their antibiotic self-administrations. The compliance of antibiotic e-diary will be calculated for each course and overall based on the dosing schedule of self-administered

ciprofloxacin and doxycycline as specified in **Figure 2**. The compliance will be summarized with the following categories: Did not enter any diary, < 50%, 50-75%, and >75%.

8.6.4 Auto-antibody Testing

Samples taken for testing for RF, ANA and anti-dsDNA antibodies and TSH assessment will be held and only be tested if medically indicated (e.g., in the event of a reported potential AESI). All test results will be provided in the data listings.

8.6.5 Pregnancy

Participant listing of FSH, and pregnancy test results (serum and urine) will be provided.

8.6.6 Urine Drug Screening and Viral Serology Testing

Urine drug screening and viral serology testing results will be provided in the participant listing.

9 INTERIM ANALYSES

No interim analysis is planned for the study.

10 FINAL ANALYSIS PLAN

A Statistical Analysis Plan (SAP) will be finalized prior to clinical database lock [to include data up to and including last subject's last in-clinic visit (Day 51; ie, four weeks after second vaccination)]. Clinical Study Report (CSR) will be generated with data up to and including last subject's last in-clinic visit (Day 51). All data after Day 51 (i.e., data up to and including last subject's 12-month safety follow up telephone call) will be included as an addendum to the CSR.

11 REFERENCES

1. CBER, Center for Biologics Evaluation and Research [Internet]. Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. September 2007. Accessed 19 Jun 2018: https://www.fda.gov/downloads/BiologicsBloodVaccines/ucm091977.
2. Maganti, L., Panebianco, D., Maes, A. Evaluation of Methods for Estimating Time to Steady State with Examples from Phase 1 Studies. *The AAPS Journal.* March 2008, Volume 10, Issue 1, pp 141–147.
3. Marcus, R., Peritz, E., Gabriel, K.R. On Closed Testing Procedures with Special Reference to Ordered Analysis of Variance. *Biometrika* (1976), 63, 3, pp. 655-60.
4. BBRC Study 762-G004690, "Two Site Validation of the Human High Throughput Toxin Neutralization Assay (htpTNA) Conducted in Conjunction with Validation Protocol VP2007-164."

5. Emergent global standard operating procedure, Production and Control of Statistical Analysis Plan (SAP) and SAP Amendments. SOP003107, version 3.0, effective on 02 Feb 2017.
6. Emergent global standard operating procedure, Statistical Oversight of Clinical Studies. SOP041684, version 2.0, effective on 18 Sep 2018.
7. Emergent global standard operating procedure, Database Lock. SOP002988, version 3.0, effective on 12 Jun 2017.
8. Emergent global standard operating procedure, Production of Tables, Listings and Figures for Clinical Study Reports and Regulatory Submissions. SOP041908, version 0.3, Ready for Approval.
9. IQVIA Operating Procedure, Statistical Principles. CS_OP_BS001 Revision 11, effective on 15 Mar 2018.
10. IQVIA Operating Procedure, Ensuring Quality in Biostatistical Deliverables. CS_OP_BS0216 Revision 2, effective on 1 Aug 2016.

12 APPENDICES

APPENDIX I TABLE/LISTING/FIGURE TEMPLATE GUIDANCE

a. Convention for All Outputs

- Unless otherwise specified, all computer-generated table/listing/figure (TLF) outputs should be produced in landscape mode. Required margins: 1inches on top and bottom and 1 inch on the left and right; required font: Courier New; and required font size: 8, at the minimal.
- Single line space is the standard for all TLFs.
- All output should have the following header at the top of the page:

  [redacted] < left adjusted> Page x of y < right adjusted>

  Protocol EBS.AVA.210 < left adjusted>

  TLFs should be internally paginated in relation to total length (i.e., page number should appear sequentially as page x of y, where y is the total number of pages in each table, listing, and figure).
- Each output should be identified by a numeral, and the output designation (e.g., Table 1) should be listed on the same line, before the title. A decimal system (x.y and x.y.z) should be used to identify tables and listings with related contents. The title is centered in initial capital characters.

  Table <Table No> Table Title
  (Study Population)

  The study population should be identified immediately following the title. Insert a blank line after the last title and before the body of the TLF content.
- Footnotes should be single spaced but separated by at least a double space from the bottom line of the table. The notes are aligned vertically by the left vertical border of the table. Footnote should be ordered as follows, if appropriate:
  - Footnote 1: source listing and/or Dictionary version, if needed
  - Footnote 2: treatment group(s)
  - Footnote 3: abbreviation footnotes (if needed): Separated by semi-colon, ended by period. One space before/after "=". Display in alphabetical order.
  - Add other notes if needed
  - Last footnote: output name, programme name, TLF version, production date/time. Insert a blank line before the last footnote.
- Column headings should be in initial upper-case characters. For numeric variables, include "unit" in column or row headings when appropriate.
- For the text within the table body and not directly from the data, only capitalize the first letter of the first word as in a sentence, and do not capitalize the first letter of each word.

b. **Table Convention**

- Decimal Places for numeric results:
  - For numeric value, display mean, median, STD, minimum, and maximum the same decimal places as reported value.
  - For TNA NF_{50}, display mean, median, STD, minimum, and maximum with one decimal point or two significant digits (e.g., min 0.032 max 18.0).
  - Geometric mean ratio will be formatted with two decimal places.
- Unless otherwise specified, CIs are two-sided with 95% confidence.
- For numerical variable data summary, align decimal point of each statistics across rows. For 95 CI, align the ',' with the decimal point of above statistics.

	Group 1 (N=xxx)	Group 2 (N=xxx)	Group 3 (N=xxx)
Parameter 1			
n	xx	xx	xx
Mean	xx.x	xx.x	xx.x
SD	xx.x	xx.x	xx.x
Median	xx.x	xx.x	xx.x
Min, Max	xx.x, xx.x	xx.x, xx.x	xx.x, xx.x

- For categorical variables, the number and percentage of responses will be presented in the form XX (XX.X), where the percentage is in the parentheses. Percentages will be calculated using a denominator of all participants in a specified analysis population (and present at the analyzed visit in by-visit summaries). The denominator will be specified in a footnote to the tables for clarification where necessary. All percentages will be rounded to one decimal place. Unless otherwise specified, the "N=xx" under each column header will represent the number of participants in that group, and all percentages below will be based on this number. Align count (integer) and first parenthesis across each row. Align decimal place of percentages, with the exception of 100% (no decimal) and zero frequencies (present as 0, no percentage).

Group 1 (N=xxx) n (%)	Group 2 (N=xxx) n (%)	Group 3 (N=xxx) n (%)
xx (100)	xx (100)	xx (100)
0	xx (xx.x)	x (x.x)
xx (xx.x)	0	xx (xx.x)
xx (x.x)	x (x.x)	xx (xx.x)
x (x.x)	x (x.x)	0

- Each level of indentation within a table takes a space of 2 characters.

c. **Listing Convention**

- Listings should be sorted by treatment group and subject numbers.
- In a listing, the subject number should be displayed only once for the subject with multiple records. If a subject's records run into multiple pages, display the subject number once for every page.
- Missing data should be represented on subject listings as either a hyphen ("-") with a corresponding footnote (" - = unknown or not evaluated"), or as "N/A," with the footnote "N/A = not applicable," whichever is appropriate.
- Dates should be printed in SAS® DATE9.format ("DDMMMYYYY": 01JUL2000). Missing portions of dates should be represented on subject listings as dashes (--JUL2000). Dates that are missing because they are not applicable for the subject are output as "N/A", unless otherwise specified.
- Time should be printed in SAS® TIME5.format ("HH:MM": 17:30). Missing portions of time should be represented on subject listings as dashes (--:30). Times that are missing because they are not applicable for the subject are output as "N/A", unless otherwise specified.

a. Graphic Convention

- Each container document will be in MS Word format.
- Graphic object output will be in <*PDF, CGM, WMF, EPS, or EMF* > format.
- Same plot produced for each treatment group will have same axis ranges.
- Define line and symbol types as below:

Table I:1 Line and Symbol Types in Graph

Attribute	Parameter	Value/Line
Line	< Treatment group 1 >	Line Type = 1
	< Treatment group 2 >	Line Type = 2
	< Treatment group 3 >	Line Type = 3
	< Treatment group 4>	Line Type = 4
Symbol	< Treatment group 1 >	Symbol = circle
	< Treatment group 2 >	Symbol = triangle
	< Treatment group 3 >	Symbol = square
	< Treatment group 4 >	Symbol = empty circle