World Health Organization

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Prequalification Team Inspection services
WHO PUBLIC INSPECTION REPORT
WHOPIR
Bio-Equivalence Study

Part 1	**General information**
Organization details	
Company information	
Name and Address of Clinical Research Site	**AXIS Clinicals** 1711 Center Avenue West Dilworth, MN 56529-0675 USA
Name and Address of Bioanalytical Research Site	**AXIS Clinicals** 1711 Center Avenue West Dilworth, MN 56529-0675 USA
Name and address of Statistical Site	**AXIS Clinicals Limited** 1-121/1, Miyapur, Hyderabad-500 090 India
Corporate address of Organization	**AXIS Clinicals** 1711 Center Avenue West Dilworth, MN 56529-0675 USA
WHO product numbers covered by the inspection/ Product names/ Study numbers/ Study titles	**WHO application no. HA781** Bioequivalence study of Dolutegravir, Lamivudine and Tenofovir Disoproxil Fumarate Tablets 50 mg / 300 mg / 300 mg
Inspection details	
Dates of inspection	18 – 21 July 2023
Type of inspection	Initial, representing a joint inspection conducted by the WHO and the Spanish Medicines Agency (AEMPS).
Introduction	
Summary of the activities	AXIS provides its services to pharmaceutical and biotechnology companies worldwide, covering a diverse range of study design and drug

	products, with experience working with various dosage forms, including oral solids. In addition, the CRO performs various types of phase 1 / pharmacology studies and generic BA/BE studies: - Single and multiple ascending dose - Pharmacodynamics - Drug-Drug interactions - Bioavailability / Bioequivalence - Proof of concept - Food effect - Gender and age Effect The clinical Research services department conducts Phase II-IV studies in oncology, CNS, ophthalmology, and Dermatology.
General information about the company and site	AXIS Clinicals LLC is a US-based CRO established in 2011 in Delaware. Initially focused on business planning, AXIS acquired the Dilworth site in late 2013 and hired its first employees. After remodeling the site in 2014, the company conducted its inaugural clinical study later that year. As a 100% subsidiary of AXIS Clinicals Ltd, a CRO headquartered in Hyderabad, India, AXIS operates four facilities in the US: - Dilworth, MN: Dedicated to conducting Healthy volunteer phase 1 studies and generic BA/BE studies. - Fargo, ND: Specializes in conducting late-stage patient trials in Dermatology. - Dallas, TX: Conducts late-stage patient trials in Dermatology and Ophthalmology. - Grand Forks, ND: Serves as a satellite site for Dilworth, facilitating screening and out-patient visits for studies conducted at the Dilworth Site.
History	AXIS underwent multiple audits by USFDA, and a comprehensive list of inspections was provided.
Brief report of inspection activities undertaken	The following scope and study-related activities were reviewed: The company's history, clinical study performance, informed consent process, ethics committee approvals and correspondence, test article accountability, dispensation and storage, processing, and handling of biological (plasma) samples collected during the study, equipment

	calibration, employee training, computer controls, and a tour of the facility. Regarding the Analytical operations, coverage was provided to firm practices, qualifications of personnel, and procedures utilized during the method validations and analytical testing. A review of the clinical study data, analytical method validation, and analytical study data was conducted, along with comparison of the source data to the study reports.
Scope and limitations	
Out of scope	Not applicable

Abbreviations

ADR	adverse drug reaction
AE	adverse event
ALCOA	attributable, legible, contemporaneous, original and accurate
BA	bioanalytical
BE	bioequivalence
BDL	below detection limit
CAPA	corrective actions and preventive actions
CC	calibration curve
CPU	clinical pharmacology unit
CRA	clinical research associate(e)
CRF	(electronic) case report form
CRO	contract research organization
CTM	clinical trial manager
CoA	certificate of analysis
CSR	clinical study report
DQ	design qualification
ECG	electrocardiogram
GAMP	good automated manufacturing practice
GCP	good clinical practice
GLP	good laboratory practice
GMP	good manufacturing practice
HPLC	high-performance liquid chromatograph
LC-MS/MS	liquid chromatography–mass spectrometry
IB	investigator's brochure
ICF	informed consent form
ICH	International Council on Harmonization

(I)EC	(Independent) Ethics Committee
IMP	investigational medicinal product
ISF	investigator study file
ISR	incurred sample reanalysis
IQ	installation qualification
LIMS	laboratory information management system
LLOQ	lowest limit of quantification
LOD	limit of detection
MS	mass spectrophotometer
MVR	monitoring visit report
NRA	national regulatory agency
OQ	operational qualification
PIS	patient information sheet
PQ	performance qualification
PQS	pharmaceutical quality system
QA	quality assurance
QC	quality control
QRM	quality risk management
SAE	serious adverse event
SAR	serious adverse reaction
SOP	standard operating procedure
STD	calibration standard
SUSAR	suspected unexpected serious adverse reaction
TMF	trial master file
ULOQ	upper limit of quantification
UPS	uninterruptible power supply
URS	user requirements specifications

PART 2	SUMMARY OF THE FINDINGS AND COMMENTS

General section

1. Organization and management

A presentation was provided explaining the activities of the organization in detail.

The CRO had a comprehensive organizational chart dated 10 May 2023, detailing key positions and the corresponding responsible individuals.

The job descriptions were defined for each employee, outlining their specific responsibilities. Random verification confirmed that the job descriptions were appropriately signed and dated by the respective staff members.

A list of signatures of authorized personnel involved in tasks during each study was available and verified, demonstrating their participation and accountability.

The principles of Good Laboratory Practices were established in the responsibilities of the test facility management. The CRO management acknowledged that, as the Principal Investigator (PharmD) was an employee of the CRO, certain responsibilities usually assigned to the investigator were held by the CRO management.

Management ensured the implementation and adherence to appropriate and technically valid Standard Operating Procedures. A well-organized historical file of all SOPs was maintained.

The company had the official working hours of 8 am to 5 pm, Monday through Friday.

The observation concerning the Organization has been adequately addressed in the respective CAPA plan.

2. Computer systems

Procedures for Computer Systems validation were implemented to ensure that computerized systems were fit for their designated purpose and underwent validation, operation, and maintenance adhering to the principles of GCP and GLP, as appropriate.

An inventory of all computerized systems on the network was readily available. Modifications to the network were documented in change controls.

A satisfactory number of computers were provided to facilitate personnel in performing data entry, data handling, calculations, and report compilation as required. These computers had adequate capacity and memory to support their intended functions.

Access to the software systems containing trial-related information was controlled. Secure and unique, individual-specific identifiers and passwords were used.

The software programs utilized for critical operations were mandated to undergo validation procedures specifically tailored for their intended purposes. The validation process aimed to ensure that the software was appropriately validated for its intended use and that its development adhered to a controlled approach in alignment with the QA system. As part of the validation process, the qualification and/or validation certificates

were required to be provided under the supervision of the user. Randomly selected software systems were reviewed to verify the process.

The specific user requirements, regulatory/guideline requirements for BE studies, the operating environment in which the system was used, and the usage of the system in the studies were considered in the Performance qualification.

The validation procedures were implemented during the installation of new versions of software. The validation process encompassed upgrades, installations, operational and performance qualification. The consistent application of risk assessment to determine the appropriate level of validation for each upgrade, installation, or patch was discussed. The focus on risk assessment aimed to adapt validation processes to meet specific change requirements while balancing thorough validation and adherence to established procedures.

SOPs for usage of each software program used to perform activities of a BE study was available.

The CRO's procedures for data entry were reviewed and discussed during the inspection. Due to the digitalization of their system, the process of data entry was amended in accordance with the respective SOP which was not applicable at the time of application HA781.

The regular backing up of electronic data was described in SOP for System Back Up and Recovery. The IT staff was responsible for creating and implementing system backup schedules for their computerized production systems, covering daily, weekly, monthly, and yearly interval backups. Each specific system under consideration had its designated backup schedule and retention period, tailored to its unique requirements. To ensure the reliability and completeness of these backups, data restoration exercises were performed to verify their integrity. The CRO utilized the specific environment for conducting the backup activities.

Observations related to the Computerized systems were adequately addressed in the respective CAPA plan.

3. Quality management

The CRO demonstrated the establishment of QA and QC systems, highlighted by written SOPs. These SOPs served as guidelines to ensure that all conducted trials and generated data were documented and reported in compliance with the protocol, GxP, and applicable regulatory requirements. Since February 2023, the CRO has started to utilize an electronic document control platform. SOPs were version controlled and only the current

approved SOP was available for use. Obsoleted copies were kept in the software and available to the authorized staff as view only. However, the version applicable for the time of study performance was only available in hard copy format.

The electronic document management system also contained the AXIS' training program to release the SOPs and training lessons to the employees, according to their job title and role in the company.

At the time of the study within the scope of inspection, some of the current computerized systems were not available or implemented. As a result, study data was recorded using paper templates and in compliance with the relevant SOPs. The company provided the inspectors with the applicable SOPs that were in use during that specific period.

The chemist requested the issuance of templates on blue colour paper for analytical procedures (analytical worksheets) using a template for "Request and reconciliation for analytical procedure copies." The controlled templates for study related to HA781 application were requested on 20 Apr 2016. Verification and release of the templates were conducted by a QA-responsible individual and the Head of Operations. Additionally, reconciliation of the templates was verified on the same form by the QC-team of the laboratory, and any unused copies were securely stored together with the rest of the study data. It was emphasized that access to the blue colour papers was strictly restricted to the Study investigator / Head of operations.

A Quality manual authorized on 11 Apr 2023 was provided. The purpose of the Quality Manual was to:
- Communicate a quality management plan
- Communicate information regarding quality procedures, control, and assurance
- Provide evidence of conformity to the National and International regulatory requirements
- Share knowledge
- Provide evidence of management's commitment to quality

The Quality Assurance department operated independently from operations, with the QA Director reporting directly to the CEO. QA personnel were responsible for verifying adherence to established procedures, ensuring trials were conducted, and data generated, documented, and reported in compliance with GCP and other relevant regulatory requirements. On the other hand, Quality Control (QC) personnel were responsible for verifying that trial-related activities met the required quality standards outlined in the study protocol and applicable SOPs.

Both in-process and retrospective QA verifications (e.g., in bioanalysis, as the samples and standards were being prepared and tested) were performed.

Observations related to the QMS were adequately addressed in the respective CAPA plan.

4. Archive facilities

The CRO maintained a secure storage facility for archiving trial-related documents, equipped with firefighting measures, humidity control system, and pest control performed twice a year or as needed. For efficient management, the CRO retained only the most recent studies onsite, while utilizing an offsite facility. The facility was audited by the CRO in May 2019.

The archiving activities were managed following the respective SOP.

Access to archive storage areas was restricted and controlled, limited only to authorized personnel. A list of authorized personnel was provided during the inspection, to be displayed at the facility.

Records of document access and return were maintained. The duration for retaining study documentation, including raw data, was defined. This timeframe was also stipulated in the contract between the sponsor and the CRO, encompassing provisions for archiving financing. During the inspection, the archiving procedures for trial-related documentation were verified through document retrieval and traceability.

5. Premises

During the inspection, on Day 3 and 4, a facility tour was conducted. The provided floor plan was utilized to compare and verify the layout of the facility.

The facilities were clean and well-organized with proper lighting, ventilation, and environmental control. Surfaces such as floors, walls, and working benches were easy to clean and decontaminate.

Clinical trials were conducted under conditions prioritizing subject safety, with suitable site selection based on potential risks. The CRO provided ample space to accommodate necessary personnel and study activities. The trial site offered adequate facilities, including laboratories and equipment.

Access to the facility was strictly controlled through keycards, with locked doors to ensure security. Emergency evacuation procedures were in place, and entries and exits from the facility were recorded.

Clinical activities took place in designated sites, including a pharmacy where investigational products were stored under controlled conditions, with access control restrictions. Appropriate entry and exit records were maintained for each visit to the pharmacy.

Laboratory premises were thoughtfully designed to suit operational needs, with sufficient space provided to prevent mix-ups, contamination, and cross-contamination. Ample storage space was available for samples, standards, solvents, reagents, and records.

Laboratory premises were designed to provide adequate protection to all employees and authorized external personnel, including inspectors, by ensuring their safety while handling or working in the presence of chemicals and biological samples.

Safety data sheets were provided to staff before conducting tests. The laboratory staff demonstrated familiarity and knowledge of material safety data sheets for chemicals and solvents they handled. Proper training was given on firefighting equipment usage, including fire extinguishers/sprinklers. Staff adhered to wearing laboratory coats and eye protection. Toxic samples were safely handled in designated safety cabinets to prevent contamination. All chemical containers were fully labelled, prominently displaying warnings like "poison" or "flammable" as needed.

Adequate insulation and spark-proofing were implemented for electrical wiring and equipment, including deep freezers and refrigerators. Staff was mindful of avoiding working alone in the laboratory, although there were instances where employees arrived early or worked alone on weekends based on workload. First-aid materials were readily available, and staff received instructions in first-aid techniques, and emergency care.

Containers containing volatile organic solvents, such as mobile phases or liquid/liquid extraction solvents, were closed with an appropriate seal. Volatile organic chemicals were handled under certified fume-hoods or air extractors, and safety and eye showers were available in the laboratory.

Premises had suitable systems in place to dispose of waste, treat fumes and protect the environment in conformance with local or national regulations.

The facility was equipped with a Diesel generator and UPS system to ensure continuous power supply. The UPS system was efficiently fed by the diesel generator, providing a reliable backup power solution. This setup contributed to maintaining uninterrupted operations, safeguarding against power outages, and ensuring critical systems' stability and functionality.

Observations related to the Premises were adequately addressed in the respective CAPA plan.

6. Personnel

A qualified and sufficient team of medical, paramedical, technical, and clerical staff was available to support the trial and respond effectively to foreseeable emergencies. Throughout all trial stages, including nights, qualified and trained personnel ensured the protection of subjects' rights, safety, and well-being, providing necessary care during emergencies. To complement the team's capabilities, contract workers were employed for specific activities.

AXIS maintained a workforce of over 130 professionals in the US at the time of inspection, with department-wise distribution as follows: 12 in Administrative, 91 in Clinical - Phase 1, 11 in Bioanalytical, and 19 in Clinical Research Services and Site Management.

The inspection performed a verification of randomly selected current curricula vitae and training records of personnel engaged in trial activities, both full-time and contract workers.

Clinical section

7. Clinical phase

The clinical phase of the studies was performed on the premises of the CRO.

The CPU / Study suits housed 228 beds across 4 study units, with one unit comprising 48 hospital beds. Accommodation facilities were equipped with systems allowing subjects to alert CRO staff in case of need, and portable alarms could be provided to individual subjects.

Washing and toilet facilities were well-maintained, easily accessible, and appropriate for the number of users. Lockable toilets were alarmed for added security.

The clinical site included registration and screening areas for subjects, ensuring privacy during informed consent processes. It consisted of a study unit, recreation space for subjects, a pharmacy, rooms for investigational product administration and sample collection, as well as facilities for sample processing and storage, including freezers, along with a dining hall for preparation of standardized meals.

Furthermore, the site's proximity to hospitals was acceptable, with two hospitals located within 4 and 6 miles, ensuring a swift response in emergencies, with medical assistance reachable within 10 minutes. The CRO contacted the general Emergency number (911) when needed.

Access to the randomization list was limited to the statistician, PI and the study's pharmacist in charge.

During the inspection, a system was implemented to shift the documentation of screening-related measurements to equipment directly connected to a designated software. It was noted that this system was not in place during the actual conduct of the study related to application HA781.

8. Clinical laboratory

A clinical laboratory, i.e., Sanford Health, was utilized for sample analysis during the clinical trial. This laboratory was accredited and performed haematological tests, urine analysis, and other specified tests in accordance with the study protocol.

To ensure full traceability and sample integrity during the study, sample labelling, receipt, storage, and chain of custody protocols were strictly followed. Recently, the CRO implemented a software system for sample management.

The CRO received information about the laboratory's analytical methods, a dated list of laboratory normal ranges, and the laboratory's accreditation certificate. Additionally, the current and signed curricula vitae of the Head of the Clinical Laboratory were reviewed.

For each subject, the laboratory generated individual reports, which were included in the CRFs. Source or raw data for all conducted tests were archived in paper formats by the laboratory. It is noteworthy that the CRO began using its own pathology laboratory in 2020.

An observation related to the clinical laboratory was adequately addressed in the respective CAPA plan.

9. Ethics

The trial received approval from the independent ethics committee before commencing any study activity. The committee's independence from the sponsor, investigator, and CRO was verified by examining the respective member list. The IEC was granted ample time to thoroughly review protocols, informed consent forms, and all relevant documentation.

Informed consent form

Study participants were provided with information both orally and in writing in English, presented at a level of complexity appropriate to their understanding.

Informed consent was obtained in writing from each subject before commencing any trial-related activities. The information provided was clear, indicating voluntary participation and the subject's right to withdraw from the study at any time without explanation. Records included the applicable details for any withdrawals from the study.

Details regarding insurance coverage and procedures for compensation or treatment in case of injury or disability resulting from trial participation were available through the Insurance policy, provided by the sponsor.

Volunteers or subjects were allowed to discuss potential side effects or reactions from using investigational products with a physician before participating in the trial, allowing them to address any concerns they might have.

10. Monitoring

The study was not monitored by the sponsor's representative/monitor. However, as per the internal procedures of the CRO, the study underwent both in-process and retrospective QA-verification.

Various audits were conducted by the QA team, with audit details and findings documented in Quality Assurance audit form for clinical and bioanalytical activities. These audits aimed to ensure compliance with applicable requirements, CRO's policies, protocols, SOPs, and other relevant guidelines throughout the study.

11. Investigators

The principal investigator (PI) held the responsibility for overseeing the clinical conduct of the study, comprising of aspects such as study design, administration of investigational products, liaising with local authorities and the ethics committee, as well as signing the protocol and final study report.

The PI was qualified as PharmD; however, physicians participated as sub-investigators to provide medical assistance when required.

12. Receiving, storage and handling of investigational drug products

The information concerning accountability of investigational products was diligently recorded. Additionally, details regarding the dispensing, administration, and reconciliation were thoroughly verified. This comprehensive documentation included specifics of the pharmaceutical product used, such as the dosage form, strength, lot

number, and expiry date. Notably, the IMP was found to be appropriately retained and not discarded. The inspectors verified the IMP and the respective documentation in the pharmacy.

The pharmaceutical products were stored under appropriate conditions, following the guidelines specified in the official product information provided by the sponsor. To ensure compliance, a temperature monitoring system was implemented to continually monitor and maintain the required storage conditions. This meticulous approach safeguarded the integrity and stability of the pharmaceutical products throughout the duration of their storage.

Randomization procedures were executed following the SOP for the generation of the randomization schedule. Comprehensive records, which included the randomization list and seed, were maintained throughout the process. The Principal Investigator (PI) initiated a formal request for the randomization list generation through the designated statistician. Prior to dispensing, the PI personally reviewed and signed the list, affirming its accuracy and integrity, before handing it over to the dispensing pharmacist. This approach was used to ensure the validity and credibility of the randomization process for the study.

An example of the label was available in the TMF. Labels were pasted onto the container to ensure that the information was not lost once the lid was removed.

To ensure accurate and verifiable administration of the Investigational Product, procedures were implemented for labelling and documenting. Each subject's receipt of the product dispensed for them was verified by utilizing labels with a tear-off portion. The label design incorporated two identical labels, with one portion pasted onto the container and the other onto the CRF at the time of dosing. Furthermore, to maintain control and prevent any potential mix-ups, the empty containers for both the test and reference investigational products were diligently labelled separately. These labelled containers were stored in a designated area within the pharmacy.

Dispensing and packaging procedures were performed in accordance with the requirements and applicable SOP.

The surface on which the product was dispensed, should be thoroughly cleaned before bringing bottles of the product into the area. Any product containers (full or empty), labelling materials, contaminants, dirt, and debris were removed from the area. A second person verified that the surface area/line was clear and clean before bringing in and opening containers of the product. The IMPs were handled with appropriate utensils. Tablets were distributed into each container in accordance with the randomization list for

the comparator or the test product as appropriate, under the supervision of the PI. The two products, i.e., Test & Reference, were handled at different times. This also applied to the labelled containers. Investigational product accountability and dispensing records were maintained. Each activity was documented at the time it was performed, including records of doses administered and returned, and record of verification by a second person of each step.

Dosing was carried out in accordance with SOP for IP administration under the supervision of the investigator and qualified staff member to whom this task was explicitly delegated in writing. A mouth check was performed, in the case of solid oral dosage forms to ensure that the subject had swallowed the IP. Dosing was documented as a part of the CRFs.

After dosing, a second responsible person verified the reconciliation of the investigational product. Samples of the product in its original container were retained for the possibility of confirmatory testing, either for up to 5 years or as specified in other agreements with the sponsor. The specific procedures for sample retention were clearly outlined and explained in the contract between the sponsor and the CRO. Additionally, any dispensed products that were not administered were also retained for further evaluation and analysis as required.

Observations related to the IMP handling were adequately addressed in the respective CAPA plan.

13. Case report forms

Randomly selected CRFs from the study were reviewed. Currently, the data collection was done electronically. However, paper CRFs were used at the time of the study related to application HA781.

The trial protocol specified the data to be collected from each volunteer. Screening-related data of application HA781 was initially recorded on respective templates and later transferred to the CRFs. The CRFs / subject binders contained all relevant data, including clinical laboratory reports and ECGs. Information on inclusion/exclusion criteria and protocol-required procedures was also documented in the CRFs. Documentation regarding IMP administration and blood sample collection was available, and the eligibility verification process was adequately documented. The CRFs followed ALCOA principles, ensuring sufficient document quality with no discrepancies or incomplete information. The inspection team readily received all requested CRFs in a timely manner.

14. Volunteers, recruitment methods

Procedures for recruiting volunteers were specified in the respective SOP, which included a description of the potential methods used by the CRO for this purpose. The CRO maintained a database at the reception area to prevent cross-participation and specify a minimum time between a volunteer's participation in different studies. Access to the database was protected with password control to ensure the confidentiality of volunteer information.

During the screening process, volunteers' identification was verified through official photo ID and birthdate. Informed consent was obtained from potential subjects for any required screening procedures related to the study in the scope of inspection to determine their eligibility for the study and participation in the research portion. The clinical trial protocol provided clear criteria for subject selection, including inclusion and exclusion criteria, as well as detailed screening procedures.

A designated database was shared among CROs within the district on a volunteer basis to prevent over-volunteering, and participation data was uploaded to this central repository.

At the time of inspection, screening activities were recorded in the respective database, while during the study, paper templates were used for this purpose. Additionally, urine samples were provided at check-in for drug testing, and alcohol testing was conducted using a device. Pregnancy tests were also performed during the check-in process.

An observation related to the Recruitment and volunteers was adequately addressed in the respective CAPA plan.

15. Food and fluids

Meals were standardized and controlled, with scheduled arrangements during the study days. The CRO managed to provide study subjects with standardized meals, snacks, and drinks, following the guidelines specified in the clinical trial protocol and as per the agreement with the catering service.

To maintain accuracy, the timing, duration, and quantity of food and fluids consumed were meticulously recorded. Prior to obtaining samples from ambulatory subjects, they were questioned about their recent food and drink intake to ensure appropriate consideration during the analysis. The Principal Investigator (PI) was responsible for designing the standardized meals.

16. Safety, adverse events, and adverse event reporting

The study was planned, organized, performed, and monitored so that the safety profile was acceptable, including to the volunteers. A medical doctor was responsible for medical decisions in the case of adverse events and notifying the relevant health authorities, the sponsor, and, when applicable, the ethics committee, specifically in the case of a serious adverse event.

At the clinical site, first-aid equipment and suitable rescue medication were readily accessible in the Emergency cart, specifically prepared for emergency situations. All treatments administered to subjects were documented on a designated template, which also included information about the unsealing of the cart for drug accountability purposes. Both the usage of medication and relevant details were required to be included in the CRF, along with the supporting documentation from the Emergency cart.

The CRO had adverse event registration, concomitant medication, and reporting forms as part of the CRF.

Bioanalytical section

The inspection primarily focused on the analytical part of Lamivudine and Tenofovir of study related to application HA781, along with its associated method validation projects. Additionally, spot checks were conducted for Dolutegravir and its corresponding method validation. The following records and activities were investigated during the inspection:

- Source documentation and raw data for the validation of bioanalytical methods.
- Analysis of subject plasma samples, including examination of the respective electronic data.
- Audit trails related to electronic data capture and handling for the bioequivalence (BE) studies.
- Results of calibration standards, quality control samples (QCs), and subject plasma samples in analytical runs, along with the chromatograms generated from these runs and Watson LIMS regression calculations.
- The preparation of analyte stock solutions, calibration standards, QCs, internal standards, and reagents.

These areas were scrutinized to ensure compliance with the established protocols and guidelines, and to validate the accuracy and integrity of the data generated during the study and validation projects.

Furthermore, chromatograms and their integration, the absence of signals in the blank samples, and the absence of any unexplained interruptions in the injected sequences were

verified. The reason for the study sample repeat analyses was reviewed. The provisions and the documentation of the ISRs were confirmed.

During the review of the study documentation, the inspection team received excellent support from knowledgeable and transparent personnel. The inspectors were granted access to the chromatography raw data on the computer through the chromatography software systems, which were networked and accessible on any in-house computer.

However, there were some challenges when accessing the audit trail files due to the use of Windows 7. As a result, the IAT (Instrument Audit Trails) could only be adequately accessed directly on the respective instrument. Overall, despite the technical issues, the inspection team was provided with sufficient cooperation and resources to conduct a thorough review of the study documentation.

17. Method development, Method validation & Analysis of study samples

The method development process was well-documented, and the use of IS was justified based on relevant literature. An analytical procedure (AP) was then established for method validation. Stable isotope-labelled internal standards were utilized in the MS methods, and K_2EDTA served as the anticoagulant for both methods.

During the method validation process, in accordance with SOP for Quantitative Bioanalytical Method Validation, a run was conducted involving the analysis of a batch containing 192 samples of QC and STD samples. The number of samples in this batch was selected to be comparable in size to those anticipated to be used during routine analysis.
In addition to QCs and STDs, the batch also included unknown samples. The sample processing for these unknown samples was reviewed and discussed, during the inspection.

The responsibility for the transfer and storage of study samples, as well as access to the DFs, was diligently managed by the Lab Management and Quality Assurance (QA) personnel, ensuring adherence to best practices and maintaining data integrity. Upon receipt, the study investigator received and securely stored the samples in the designated DFs. Any subsequent transfer of samples from one DF to another was recorded on the same template log, assuring complete traceability and transparency in the sample transfer. The transfer and usage of samples for sample processing and extraction steps were however only recorded on the analytical sheets.

QC and STD samples were prepared and stored in individual vials, intended for one-time use only. After usage, any remaining QC/STD samples were discarded. Both the QCs and STDs were stored together with aliquot I, further streamlining the workflow and enhancing sample management processes.

Sample processing was documented using specific forms (blue analytical sheets). Prior to sample analysis of study related to application HA781, the Project Specifications Form was prepared. The analytical sheets, supervised by the QC-team, were printed on blue paper. Additionally, a note to file was included to record any unexpected activity during sample processing, when applicable.

Data supporting the stability of the samples under the stated conditions and storage period was available before the commencement of the studies, except for long-term stability data, which was conducted before the issuance of the study reports.

The review of the method validation encompassed selectivity, matrix effect, and calibration curve during the inspection. The performance of precision and accuracy testing (P&A), sensitivity, autosampler carry-over, dilution integrity, and various stability parameters (freeze-thaw stability, stock solution stability, and reference standard storage stability) were also verified based on the submission provided pre-inspection. Partial validation was conducted in accordance with the required criteria.

For the analytical method validation, the matrix used was the same as the matrix of the study samples, and it included the anticoagulant K_2EDTA. The plasma purchase documentation underwent thorough review and discussion. This documentation covered aspects such as receipt, storage, retrieval, preparation, and consumption of the pooled plasma, ensuring evaluation and confirmation of the quality and suitability of the analytical matrix

For the preparation of STDs and QC samples, two separate stock solutions were prepared, ensuring that they were considered equivalent after undergoing a thorough evaluation.

In each analytical run, a comprehensive set of samples was processed simultaneously. This included the STDs in both the beginning and end of the run to ensure bracketing, QC samples interspersed throughout the run, and subject samples. The exact sequence of processing was precisely defined and thoroughly documented. All samples collected from a given subject during all trial periods were analyzed within the same run.

The acceptance criteria for the analytical runs were meticulously verified by reviewing various parameters using a software system, including analytes' retention time, the accuracy of calibration standards and QC samples, peak integration, and Internal Standard (IS) peak areas. These criteria were evaluated in accordance with the applicable SOPs, such as procedure for Sample Analysis (Chromatographic) and for the Integration of Chromatographic Data.

Before the initiation of runs on each day, a system suitability test (SS) was conducted to ensure the proper functioning and performance of the instrument. However, if there were consecutive sample analysis or method validation runs on the same instrument, the system suitability test was only performed before the first run, as outlined in the SOP which was valid during the study period. This approach assured that the instrument was adequately calibrated and ready for accurate and precise analysis throughout the day's runs.

During peak integration, the chemist relied on the parameters established during the method validation. In some instances, the chemist deviated from these parameters to ensure proper integration of chromatographs, as for example observed in Run 13 and Run 12 for Tenofovir. During the inspection of randomly selected runs, the inspectors noticed that changes were made to the splitting factor, noise, or retention time. Importantly, these changes were applied to the entire batch, introducing consistency in the data processing.

Out of the first 1000 samples, 10% were utilized for Incurred Sample Reanalysis (ISR). For subsequent samples exceeding 1000, at least 5% were selected for ISR. The samples chosen for ISR were carefully selected to have concentrations around C_{max} and during the elimination phase, ensuring representative analysis. The acceptance criteria for ISR were explicitly defined in the SOP for Incurred Sample Reanalysis, which was applicable during the study period.

The CRO had a standard practice of conducting a Pre-study sample run comprising Calibration Curves (STDs) and Quality Control (QCs) samples before the study initiation. This practice ensured that the STDs and QCs were accurate and within the desired range, providing a robust starting point for the study.

An observation related to the Sample analysis was adequately addressed in the respective CAPA plan.

18. Sample collection, storage, and handling of biological material

Samples (blood plasma) were collected according to the clinical trial protocol and information provided to volunteers. They were handled, prepared, transported, and stored in line with SOP for Sample Handling. The samples were then directly transferred from the processing room to the Deep Freezer room, located on the opposite side of the corridor, and kept under the custody of the study investigator.

Sampling times, including any deviations from the prespecified schedule, were recorded. These deviations were considered during the calculation of the pharmacokinetic parameters.

Samples were clearly labelled to ensure accurate identification and traceability. Storage conditions, including freezer temperature, were monitored, controlled, and documented during storage using a digital monitoring system. Detailed records of sample storage and retrieval were maintained. To enhance security, samples were duplicated into aliquots and shipped and stored separately.

Per SOP and the agreement with the sponsor, study samples, QC & STD samples, and pooled matrix were disposed of accordingly. The preparation and planning of STDs and QCs for various runs were documented using a designated form. Additionally, a solution tracker was provided to record the usage of specific STDs and QCs for each batch. Biological samples were required to be retained for a minimum of three months after delivering the bioanalytical results to the sponsor.

Observations related to the Handling biological samples were adequately addressed in the respective CAPA plan.

19. Data processing and documentation

Integration settings were science-based and justifiable. The smoothing factor was kept low enough not to mask possible interferences and changes in peak geometry.

The criteria for acceptance and exclusion of STD and QC samples, as well as batch acceptance, were clearly defined in the applicable SOP. The source data for all the analytical runs contained all information about the original first evaluation of runs (containing all calibration samples) when the analysis was repeated. The calibration range was adequately truncated. Internal standard variations were trended and used as part of the verifications of result validity.

Audit trails were activated on all analytical instruments before, during, and after the method validation and the sample analysis of the study HA781.

All original analytical raw data (e.g., calculations, chromatograms, and their associated audit trails) were documented in a manner that ensured traceability concerning the sample number, equipment used, date and time of analysis, and the name(s) of the technician(s). All audit trail files were retained (e.g., results table audit trail, project audit trail, and instrument audit trail).

Each data point was traceable to a specific sample, including sample number, time of collection of the sample, time of centrifugation, time when the sample was placed in the freezer, and time of sample analysis, to be able to determine whether any aberrant results might have been caused by sample mishandling.

20. Good laboratory practices

On Day 4, a facility tour was conducted to assess the laboratory's suitability concerning arrangement and safety. Throughout the bioanalytical phase of the bioequivalence studies, the general principles of Good Laboratory Practice were adhered to. This involved implementing an established and appropriate Quality Assurance system to ensure the integrity and reliability of the study results.

Deep freezers for storage of the samples and refrigerators for storage of the Reference standards were qualified, calibrated, and maintained. There was an alarm system associated with the digital temperature monitoring system to trigger call notifications to the responsible staff. The automatic alarm system was tested during the inspection to verify its proper functionality. The daily monitoring and all the alarm checks were documented.

To verify the qualification of the Deep Freezer, the temperature mapping conducted on 23 Oct 2019 was reviewed. This qualification was applicable for five years. During the review, particular attention was given to verify the hotspot identification and the location of the respective temperature sensor. The sensors were positioned inside a container containing glass beads. In terms of maintenance and repair, the appropriate considerations were made to ensure the proper transfer of samples to equivalent storage units when necessary.

Balances, other measuring devices, and equipment and instruments used during the conduct of a trial were periodically calibrated and verified before use to be fit for their intended purpose.

The operation, use, calibration, checks, and preventive maintenance of equipment were described in the respective SOPs. Records were maintained. These activities were verified by random review of the equipment used in study-related activities, e.g., the LC-MS/MS instrument used for the period of study HA781. Equipment and its components were labelled with the respective ID number, date of calibration, and date of next calibration. The equipment usage was documented in the analytical sheets, as well as the respective templates for the instrument usage.

Chemicals, reference substances, reagents, solvents, and solutions were labelled to indicate identity, purity, concentration when appropriate, expiry date, and specific storage instructions. Information concerning the source, preparation date, and stability was available on the label or the CoA.

Observations related to the Good Laboratory Practices were adequately addressed in the respective CAPA plan.

Pharmacokinetic, statistical calculations and reporting section

21. Pharmacokinetic, statistical calculations

On Day 4, a presentation was conducted to explain the respective activities of the PK department. During this presentation made by the statistician, it was highlighted that the PK analysis for the bioequivalence study was based in India. One of the employees in the US office served as a liaison between the medical writing team in the US and the statistician team in India. The PK analysis at the CRO's PK department for the BE study followed specific procedure.

Randomization requests were received via email or network drives from the Principal Investigator (PI), pharmacist, or designee. Randomization was generated based on the IRB-approved study design, using SAS software application in accordance with the respective SOP. It was then shared with QA for review through email or network drives. After QA review, the randomization was shared with the PI for approval, and once approved, the PI signed the document. The biostatistician maintained a PI-signed copy for future reference with restricted access.

The statistical model underlying the primary BE analysis was explicitly stated in the study protocol. Calculations were performed using a dedicated software system, and as an additional quality check, a second qualified individual cross-verified the input data values in accordance with the applicable SOPs.

In terms of the PK analysis specifics, Phoenix WinNonlin was employed as the software for conducting the analysis of study HA781.

At the time of study HA781, data transfer for time deviations was conducted via email in PDF format. Similarly, bioanalytical concentration data was also transferred through email for the PK analysis. The clinical team was responsible for entering the data. A formal Quality Control team was not in place, and the data was subsequently sent to the Quality Assurance (QA) team for verification. QA performed a thorough check, verifying 100% of the data through demographic and chromatogram analysis.

Currently, bioanalytical data generated by the BA department is shared through email with the statistician. Time points for analysis are directly exported from the designated software system, ensuring streamlined data handling and analysis processes.

22. Study report

The process of study report writing was verified during the inspection. Procedures were established to ensure the quality and integrity of the study report. No discrepancies were identified between the results stated in the report and the original (raw) data.

The study report included a report on the bioanalytical part of the trial, including a description of the bioanalytical method used and a report on the validation of this method. The Principal Investigator approved CRFs before data transfer to the statistical department. Similarly, Bioanalytical reports were approved by responsible staff and management. Furthermore, audit reports were made available prior to the release of the final study report.

Miscellaneous	
Samples taken	N/A
Assessment of the CRO master file	A CRO Master File was provided during the inspection.
Annexes attached	N/A

Part 3	**Conclusion – outcome of inspection**

Based on the areas inspected, the people met, and the documents reviewed and considering the findings of the inspection, including the observations listed in the Inspection Report, as well as the corrective actions taken and planned, the study was considered to have been conducted at an acceptable level of compliance with WHO GCP/GLP/BE guidelines at ***AXIS Clinicals***, located at ***1711 Center Avenue West, Dilworth, MN 56529-0675; USA***.

All the non-compliances observed during the inspection that were listed in the complete report as well as those reflected in the WHOPIR were addressed by the CRO, to a satisfactory level, prior to the publication of the WHOPIR.

This WHOPIR will remain valid for three years, provided that the outcome of any inspection conducted during this period is positive.

Part 5	**List of guidelines referenced in the inspection report**

1. Guidance for organizations performing in vivo bioequivalence studies. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fifties Report Geneva, World Health Organization, 2016 (WHO Technical Report Series, No. 996), Annex 9. ***Short name: WHO BE guidance*** or ***TRS996 Annex 9***

2. Good clinical laboratory practice (GCLP), WHO on behalf of the Special Programme for Research and Training in Tropical Diseases. Geneva, 2009

Short name: WHO GCLP

3. Guidelines for good clinical practice for trials on pharmaceutical products. WHO Technical Report Series, No. 850, 1995 (pp. 97–137).
   Short name: WHO GCP

4. Handbook – Good Laboratory Practice (GLP): quality practices for regulated non-clinical research and development – Annex I: The OECD Principles on GLP, 2nd ed., 2009. ***Short name: OECD GLP***

5. Standards and operational guidance for ethics review of health-related research with human participants. Guidance Document. Geneva, World Health Organization, 2011.
   Short name: WHO Ethics Committee Guidance

6. Guidelines for the preparation of a contract research organization master file, WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-fourth Report. Geneva, World Health Organization, 2010 (WHO Technical Report Series, No. 957), Annex 7.
   Short name: WHO CROMF Guidelines or ***TRS No. 957, Annex 7***

7. Model guidance for the storage and transport of time-and temperature-sensitive pharmaceutical products. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Forty-Fifth Report. Geneva, World Health Organization, 2011 (WHO Technical Report Series, No. 961), Annex 9.
   Short name: WHO storage and transport guidance or ***TRS 961 Annex 9***

8. Glove use information leaflet, Patient Safety, Save lives clean your hands. Geneva, World Health Organization, 2009 (revised).
   Short name: Glove use information leaflet

9. Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. Republication of multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability. WHO Technical Report Series No. 992, Annex 7 with a new appendix 2. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fifty-first Report Geneva, World Health Organization, 2017 (WHO Technical Report Series, No. 1003), Annex 6.
   Short name: TRS 1003 Annex 6

10. Good chromatography practice. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fifty-fourth Report. Geneva, World Health Organization, 2020 (WHO Technical Report Series, No. 1025), Annex 4.
    Short name: WHO TRS No. 1025, Annex 4

11. Guideline on data integrity. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fifty-fifth Report. Geneva, World Health Organization, 2021 (WHO Technical Report Series, No. 1033), Annex 4.
Short name: WHO TRS 1033, Annex 4

12. Declaration of Helsinki, World Medical Association Declaration of Helsinki, Ethical principles for medical research involving human subjects, Bulletin of the World Health Organization, 2001 (79(4)).
Short name: Declaration of Helsinki

13. Bioanalytical Method Validation and Study Sample Analysis M10, ICH Harmonised Guideline, Final version, Adopted on 24 May 2022
Short name: ICH M10

14. Good Manufacturing Practices: Guidelines on validation. WHO Expert Committee on Specifications for Pharmaceutical Preparations. Fifty-Third Report Geneva, World Health Organization, 2019 (WHO Technical Report Series, No. 1019), Annex 3.
Short name: WHO TRS No. 1019, Annex 3