



WHO PUBLIC INSPECTION REPORT

(WHOPIR)

Contract Research Organization

Part 1: General information

WHO product numbers covered by the inspection	MA85 Artesunate/ amodiaquine 100/270 mg tablets
Study number	Study Code No. CP1103 (CP1104 v04)
Title of the study	Study CP1103: this study is an open-label, randomized, single-dose, two-sequence, four-period, crossover, replicate design (2 x 4), bioequivalence study conducted in 31 healthy Chinese volunteers under fasting conditions.
Clinical Part of the study: Name and address of the organization	Shanghai Xuhui Central Hospital Phase I Clinical Research Unit and Bioanalytical laboratory. Shanghai Xuhui Central Hospital, 966 HuaiHaiZhong Rd, Shanghai China 200031
Bio-analytical laboratory: Name and address	Same as above.
Date of inspection	22 to 25 October 2012

Part 2: Summary

General information about the site(s)

The Shanghai Xuhui central hospital (referred to as “Shanghai Xuhui” hereafter) was founded in 1934 with more than 800 beds and 810,000 outpatient visits annually. It has 31 therapeutic departments. The phase I clinical research unit was established in 2000. It received a SFDA certificate to conduct clinical trials in 2006. The certificate was renewed in February 2012.

Vivo Development Ltd (referred to as “Vivo” hereafter), established in 2005, is a Contract Research Organization (CRO) specialized mainly in phase I clinical studies. It formed an alliance with the hospital in 2008. It was responsible for the drafting of the protocol and associated documents (eg. informed consent forms, etc.) as well as the management of the study, monitoring, statistical analysis and reporting.

The activities at the site were divided between the old facility and the new facility. The old facility was established in 2000, on the 13th floor of building 2, with 28 beds and areas for patient screening, drug administration, blood sampling, drug storage,

bioanalytical laboratory, document storage area and an emergency rescue room. The new facility was established in 2009 and is located on the 20th floor of building 1 and contained 36 beds (28 +8 extra beds) at the time of the inspection.

In the bioanalytical laboratory, there were 6 tandem liquid chromatography - mass spectrometry (LC-MS/MS) systems (two API3000, API3200 and API5500 and two API4000 models) (note: only 5 were described in the company presentation). Approximately 48 biostudies have been conducted from 2006 to date. Hematology, microbiology and biochemistry testing for studies performed at the Shanghai Xuhui site were performed by the hospital.

History of WHO and/or regulatory agency inspections

This was the 2nd WHO inspection of this site. The 1st inspection was performed in June 2010 and found the study under inspection, for a different product, to have been conducted at an acceptable level of Good Clinical Practices (GCP) and Good Laboratory Practices (GLP) although a relatively high number of observations had been found.

Focus of the inspection

This was the 2nd WHO inspection of this site. The 1st inspection was performed in June 2010 and found the study under inspection, for a different product, to have been conducted at an acceptable level of GCP and GLP although a relatively high number of observations had been found.

The inspection covered all the sections of the WHO GCP and GLP texts, including the WHO guidance for organizations performing in vivo bioequivalence studies.

Inspected Areas

Day 1

After introductions and a short company presentation, the inspector performed a tour of the facilities which included: a visit of the sample preparation room, deep freezer room and LC-MS/MS room.

The procedure for routine maintenance of the deep freezer was explained by the site staff.

Documents reviewed included the following:

- Procedure on the use and maintenance of computer systems.
- Documentation of monthly backup and password protection lists per user.
- Procedure on record retention and maintenance of archives.
- Description of procedures for data transfers and management.
- Contractual agreement between Vivo, Shanghai Xuhui, and the sponsor: Vivo is responsible for communication with WHO PQ, responsible for the monitoring of the clinical trial and verification that the data are generated, documented and reported in accordance with the clinical study protocol and

GCP GLP requirements. Vivo is also responsible for the pharmacokinetic, biostatistical analysis and safety efficacy conclusions. According to the contract, any remaining Investigational Medicinal Products (IMPs) are returned to the sponsor at the end of the study. The reserve sample was returned to the sponsor for the study under inspection.

- Randomization list generated by Vivo and given to the Principal Investigator (PI) and IMP administrator.
- Monitoring: it is documented by a monitoring report before the study and every time dosing is done as well as the close out, for a total of 6 visits.
- Data archival: hardcopies are archived in the Trial Master File in the local archive room while electronic copies are archived by the sponsor and Vivo. For archiving at Xuhui, hard copies of logbooks for instance, were kept for up to 1 year in the unit and then afterwards in the archive room of the hospital for 5 years.
- The following aspects of High Performance Liquid Chromatography (HPLC) related procedures were examined:
 - Procedure on integration of chromatographic peaks. This procedure stated that the analyst may change the integration parameters by manual integration, but this should be avoided as much as possible. The procedure also stated that if the peak is manually integrated, the bioanalyst should document that the peak was manually integrated and the reason. This was seen to be done in practice hence the standard operating procedure (SOP) was adequately applied.
 - Verification of the absence of any significant or unexplained time gaps during runs or in between analytical runs for subjects.
 - Verification of system suitability runs.
 - System security settings and user access rights (deletion, renaming and rewriting of files, changes in system date and time).
 - Settings of audit trail parameters.
 - Variability of the internal standard response and slope.
 - Exclusion of calibration samples.
 - Nominal concentrations of the calibration samples, entered into the Analyst software to calculate concentrations.
 - Settings of the LC-MS/MS system for one sample in each run, and comparison of these settings with those listed in the method SOP.

Day 2

Inspectors reviewed analytical data relating to the study directly on the 2 LC-MS/MS systems which were used at the time of the study. This included:

- Verification of the calculated concentrations vs. nominal concentrations and percentage difference using documentation relating to the preparation of the calibration and quality control (QC) samples used for the main validation experiments (precision and accuracy, long-term stability, etc.) and of the stock and working solutions used to prepare these samples. Their nominal concentrations were recalculated.
- Verification of whether calibration curve (CC) and QC samples were prepared from separate stock solutions: this was found not to be the case. This was verified by comparing two stock solutions (or subsequent diluents) prepared

from different weighing. The mean difference (n=6) between the two stock solutions should be within 5% of each other.

- Matrix consumption records and other documentation relating to the blank matrix used during method validation (individual lots of plasma and pooled matrix). The number of successive freeze/thaw cycles of the blank matrix and plasma pool was not reviewed due to time constraints. However, from the results obtained (very few QCs were actually rejected), and peak shape variability being low, this did not appear to be a significant issue.
- The dates of each preparation were verified vs. the deep freezer logbook - the time between removal of plasma from the freezer and the time of preparation was reasonable.
- The dates of some randomly selected preparations were verified vs. the deep freezer logbook - the time between removal of plasma from the freezer and the time of preparation was found to be reasonable in general.

Day 3

Inspectors proceeded with the inspection of statistical and the clinical portions of the study. This included a review of the following documents:

- QA and QC checks (QC checks on the SAS program was provided).
- Volunteer registration database (this included a verification of whether volunteers could be readily retrieved from the database using their fingerprints).
- Informed consent forms and verification of their contents vs. the innovator Summary of Product Characteristics
- Screening and examination rooms.
- Electrocardiogram (ECG) room.
- Clinical pharmacology units on the 20th and 13th floors.
- Custodian area.
- Alarms and their linkage to the beds and restrooms.
- Intensive care units (ICUs).
- Archive of the CRO on the 20th floor.
- Pharmacy.

Day 4

Remaining items from the previous days were inspected.

This included documents and procedures pertaining to the Independent Ethics Committee (IEC), as well as:

- Qualification records of the ultra-deep freezers used in the study.
- Ultra-deep freezer storage and withdrawal records.
- Electronic bioanalytical data for amodiaquine.

2.1. PROVISIONS AND PREREQUISITES FOR A CLINICAL TRIAL

A 4 period study replicate design was used so that for each subject, intra subject variability could be characterized by administering the test or reference product twice to the same subject.

There were 5 subjects discontinued either at their own will or because the PI had intended for them to withdraw.

Regulatory requirements, justification for the trial and ethical principles were considered to be acceptable overall.

2.2.THE PROTOCOL

Vivo was responsible for the writing of the protocol. More details on the approval of the protocol by the IEC are included in the next section (“2.3 Protection of Trial Subjects”).

The following table describes deviations from the protocol:

Subject #	Sequence	Period	Treatment	Deviations	Evaluations
R04	2	3	Reference Product	In period 3, the 8hr post-dose blood sample was delayed by 3min.	For more accurate calculation of AUC values, this deviation was incorporated into the PK analysis. It is more important for AQ, as concentrations of AS has fallen below LLOQ at 8hr post-dose. This was a minor deviation and considered to have little or no impact on the results of the study.
R12	1	3	Test Product	In period 3, the 10, 20, and 30min post-dose blood samples were delayed by 3, 4, and -1min, respectively.	For more accurate calculation of AUC values, these deviations were incorporated into the PK analysis. This was a minor deviation and should have little or no impact on the results of the study.
R15	1	4	Reference Product	In period 4, the 8hr post-dose blood sample was delayed by 4min.	For more accurate calculation of AUC values, this deviation was incorporated into the PK analysis. It is more important for AQ, as concentrations of AS has fallen below LLOQ at 8hr post-dose. This was a minor deviation and should have little or no impact

Subject #	Sequence	Period	Treatment	Deviations	Evaluations
					on the results of the study.

2.3. PROTECTION OF TRIAL SUBJECTS

Subjects were entered into a database for each study. Information entered included age, mobile phone number. ID was verified by identity card and supported by fingerprints. Pictures of subjects on both ID card and in person were saved in the system .

There are other CROs in the Shanghai area, but no common system for sharing information between them. The CRO placed advertisements for recruitment of volunteers on their website and relied on word to mouth as well. Subjects were mostly from the hotel and restaurant service industry because of the flexible schedules which would accommodate their participation in biostudies. There were approximately 300 subjects registered in the database.

For the MA85 study, there were 37 subjects who were enrolled and 36 received test or reference drug according to the randomization table, one subject was a backup and did not proceed to the study.

ECGs were taken for subjects as part of the screening process. They were saved in a PC database using ECG lab (powered by DM software Inc). The system was tested to see if subject details can be changed. This was found to be possible however, the date of obtaining of the ECG could not be changed.

Blood withdrawals for the purpose of screening was done in a dedicated area. There were no Vacutainer adaptors used, but instead a needle coupled to disposable plastic tubing which is connected directly to the Vacutainer.

Coagulation inside the cannula tubing is prevented using sodium heparin solution. There were no written instructions as to how this should be done, although it was stated that only one syringe was used per bottle and that it was kept connected with the bottle.

There was no test for alcohol at check in. This was stated not to be necessary in this case due to the low risk of alcohol abuse in the area. Since the subject appeared more than 12 hours before the study, this was not found to be an issue because access to alcohol would be prevented during this period. These measures were supplemented by the questionnaire's questions regarding alcohol consumption which was used to screen out subjects suffering from dependence to alcohol and other substance.

If a subject suffers an AE, the sponsor, according to the contract, will reimburse reasonable and necessary medical expenses incurred by subjects as a direct result of the treatment or adverse reaction. However, there was no statement with regards to

expenses arising from the misconduct or negligence of any agent or employee of Shanghai Xuhui hospital. This was requested to be done. A medical accidental insurance for the hospital was provided.

With regards to the Shanghai Xuhui Central Hospital Ethics Committee (IEC):

A record of the documentation submitted on 21 December 2011 and approved on January 6, 2012 was reviewed as well as the amendments (approved on February 2, 2012). It included:

- Approval documentation from the SFDA.
- Study protocol (original and amended versions).
- Informed consent forms (ICFs) (original and amended versions).
- Investigator's manual.
- Certificates of analyses of the medicines.
- Qualifications of the sponsor.
- GMP certificate production licence and drug registration certificate.

The IEC meeting took place at the CRO and only discussed the 3 period design trial protocol. There were questions about the proposed washout period and screening time as well as the ICF. The IEC committee proposed that there would be a better description of medical terms, e.g., Type I allergic reaction, washout period, screening, and add a flow chart for the study. The list of documents. The IEC committee was given approximately 2 weeks to review the documents prior to the meeting. Deficiencies were found with regards to the independence of the ethics committee and potential conflicts of interest of one participant. This was resolved in the CAPAs submitted by the company.

2.4. RESPONSIBILITIES OF THE INVESTIGATOR

Shanghai Xuhui had fulfilled its responsibilities as investigator. Biological samples were kept until the product was approved by the WHO and thus were still at the site at the time of the inspection.

2.5. RESPONSIBILITIES OF THE SPONSOR

The sponsor delegated part of its responsibilities to Vivo.

The sponsor performed an audit of the dossier prior to its submission and was present during the inspection. Guilin was responsible for supplying the IPs to Shanghai Xuhui and for testing their quality and recovered them at the end of the study.

2.6. RESPONSIBILITIES OF THE MONITOR

The study was monitored by the Vivo Development CRA. She monitored dosing and several other activities. She came on 21 February, 28 February, 6 March, 13 March and 31 March 2012.

2.7. MONITORING OF SAFETY

This was adequately and thoroughly performed.

2.8. RECORD-KEEPING AND HANDLING OF DATA

This was the responsibility of Shanghai Xuhui in collaboration with Vivo Development (e.g. statistical analysis and reports).

2.9. STATISTICS AND CALCULATIONS

Statistical data and the commands used were verified. Version 9.2 of SAS[®] was used. The results obtained matched those submitted in the dossier.

Randomisation was done using a Test/Reference/Test/Reference (TRTR) or Reference/Test/Reference/Test (RTRT) (there was only 2 possibilities) in line with the recommendations of the US FDA guideline on statistical calculations. 36 subjects were enrolled and 31 completed the study as per protocol.

The test drug for artesunate was generally lower than the reference drug but within acceptable limits.

Concentration data was received as excel data and transferred into SAS format by the biostatistician. The data used was dated 12 to 23 March 2012.

Time point deviations were entered by hand in the SAS format sheets by the biostatistician.

2.10. HANDLING OF AND ACCOUNTABILITY FOR PHARMACEUTICAL PRODUCTS

Records were examined for the test and reference product and the verification of accountability was reviewed. The dispensing form dated 15 September 2010 was used. It included verification of line clearance.

Accountability of products was recorded in the “IMPs accountability form”. There were 300 tablets of the reference product batch 5355 received by Shanghai Xuhui. The number of containers received was not documented in this form. 162 was dispensed at once for the 4 periods and 166 were remaining in the end and were returned to the sponsor. Once dispensed, these were kept in the same storage cabinet as where previously stored. For the test, there were 180 tablets received. 162 dispensed, 46 returned.

Improvements were seen compared to the last WHO inspection by establishing an SOP for shipping and handover of IMPs to ensure that requirements of storage conditions are met during shipping and document the process from product release to the clinical site, including the in transit period. Only minor temperature deviations were observed for this particular study.

The management of IMPs for clinical studies by the Phase I unit was also reported to have been improved by the updating the SOP for the management of IMPs.

Improved control of IMPs by designating and IMP administrator and access control of the IMP storage room.

It was noted that there was:

- Improved drug accountability, shipping receiving and dispensing.
- Improved the dispensing process with line clearance between test and reference product with laminar flow hood and HEPA filtration.
- Verification of the matching information on the IMP bag and source document.
- Improved environmental control of the IMP storage room.

QA performed verification of temperature and humidity, line clearance, dispensing practice (adequate protection) and verified the time that dispensing commenced as well as that the sequence was accurately followed.

The sub investigator performed a check of the documents used in the study, protocol, which was supplemented by QA. It included a verification of the printing of the labels.

The dispensing room is continuously monitored for temperature and humidity. It was access controlled but there was no log of exit or entry.

CRFs were examined against the randomization list in SAS for randomly selected subjects.

Certificates of analyses for both the test and reference product were provided.

2.11. ROLE OF THE DRUG REGULATORY AUTHORITY

The Drug regulatory authority had approved the trial prior to its taking place and had inspected the site several times.

2.12. QUALITY ASSURANCE FOR THE CONDUCT OF A CLINICAL TRIAL

The SOP systems for Xuhui and Vivo are now independent and govern their own respective activities. There was confusion as to the respective organisation's roles during the last inspection.

The QA auditors at Xuhui were responsible for all in process audits for clinical studies. There were 13 SOPs established, the group is dedicated to QA, performs QA audits regularly according to an audit plan, performs QA audits for both clinical and bioanalytical activities.

QA was present during dispensing (see description under Handling and accountability of pharmaceutical products section).

With regards to the bioanalytical data, daily results were printed from Analyst. 100% of data were verified by a QC staff, this practice was described in an SOP. If

abnormal values are found, they will go back to the raw data. The results from Analyst are copied in the excel spreadsheet. This was done on 9 April 2012 for artesunate and on 23 March 2012 for amodiaquine. This data was verified by QC and captured in a QC audit procedure before being passed on to QA. The QA check was documented in a corresponding report.

BIOANALYTICAL

Key study facts

Amodiaquine sample bioanalysis was performed from 27 February to 23 March 2012. Artesunate sample bioanalysis was performed from 24 February to 4 April 2012 on a different HPLC-MS/MS system. Bioanalysis was performed in September 2012. From 20 February to 16 March 2012; AS and DHA was analysed in plasma from 20 February to 16 March 2012, while AQ was analysed in whole blood during the same period using different HPLC-MS/MS equipment. Sodium heparin was the anticoagulant in use for sample collection at all times (see observation under “Part 5” regarding the lack of documentation of this). Only 1 HPLC-MS/MS was used per method for both sample analysis and validation.

Method development

AS and DHA were extracted from human plasma samples using a protein precipitation method. AS and DHA were analyzed by LC-MS/MS, using stable isotope labelled internal standards for each analyte ([2H]-AS and [13C, 2H4]-DHA). Both analytes were quantified on LC-MS/MS systems with reverse phase LC and an isocratic elution procedure, and detected by a triple-quadrupole mass detector in positive ion mode with electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode. Peak area ratios of AS and DHA over those of the corresponding internal standard were used to quantify AS and DHA plasma concentrations. Calibration curves were constructed by the LC-MS/MS software Analyst 1.5.1 using quadratic regression curve fitting for both AS and DHA.

AQ was extracted from human whole blood samples using a protein precipitation method. AQ was analyzed by LC-MS/MS using a deuterium labelled internal standard ([2H10]-AQ). AQ was analyzed on an LC-MS/MS system with a reverse phase LC system and a gradient elution procedure, and the analyte and IS were detected by a triple-quadrupole mass detector in positive ion mode with electrospray ionization (ESI) in multiple reaction monitoring (MRM) mode. Peak area ratios of AQ over those of the internal standard were used to quantify AQ blood concentrations. Calibration curves were constructed by the LC-MS/MS software Analyst 1.5.1 using linear regression curve fitting for AQ.

Method validation

The original validation was performed in July-August 2011 for the first study performed. Matrix effects were then repeated in December 2011. Partial validation was performed in February and March 2012. Another partial validation was conducted for hemolyzed plasma of AS/DHA in April 2012.

The availability of different instrument models within the same laboratory raised the question as to whether cross-validation or revalidation of the methods would be necessary. Since the laboratory used the same system for AS, DHA and AQ, this was not found to be a necessity except to cover the fact that a significant amount of time had elapsed between the original validation and testing, hence calibration of the same instruments may have changed. This was covered by the partial revalidation which was performed.

Auto-sampler platform stability: the validation report did not include the temperature setting of the auto-sampler but these are recorded in Analyst method settings for the run. Samples were demonstrated to be stable over the maximum period of storage encountered during runs, on the auto-sampler.

Partial revalidation for AS/DHA which had been performed between 10 February 2012 to 30 March 2012 included a selectivity experiment performed at the LLOQ of 1 ng/mL and at the high QC level of 750 ng/mL for AS; and LLOQ of 3 ng/mL and at the high QC level of 2250 ng/mL for DHA. The selectivity experiment chromatograms, although obtained on blank plasma from 6 individuals, did not include any peaks with the poorly resolved peak that interfered with the integration of artesunate at low concentrations. Since concentrations where the poorly resolved peak was seen were quite low, this is not estimated to have an impact but should nevertheless have been documented by the company (see observation under “Part 5”).

Preparations of samples

All blood samples were divided into two parts, part one centrifuged at 4°C within 30 minutes of collection to obtain plasma for the analysis of AS and DHA, while part two samples were stored directly in duplicate for the analysis of AQ. Plasma and blood samples were stored in polypropylene tubes at below –70°C until analyzed.

The duration of storage of biological samples was not specified in the contract or documentation but was addressed by the SOP on Biological Samples – Receipt, Handling, Inventory, Tracking and disposal.

Preparation of stock and other solutions

Blank plasma lots were obtained at the hospital. Documentation of the anticoagulant used was requested but was not available. It is noted that 100 mL volumes were taken for storage from each volunteer during withdrawal of blank plasma.

Handling of data

ISR was performed on 266 samples for artesunate, corresponding to 11.0 % of total samples, 268 for DHA (11.1%) and 268 for amodiaquine (11.1%).

The batch acceptance criteria used had been found to be acceptable by assessors during dossier review and practices were confirmed during the inspection. Repeated analysis was adequately justified (samples above ULOQ, malfunction of equipment). The results from the incurred samples reanalysis were found to be acceptable.

Due to stability issues, the analysis of samples for the determination of AQ was made from each period. For AS and DHA, analysis was made for each subject from period 1 and 2 together (TR or RT) and for period 3 and 4 together.

The company's method of performing manual integration was acceptable and was done in a controlled fashion, accompanied by a justification and was found to use good scientific judgement and common sense in those examples that were examined during the inspection.

Some observations were raised with regards to the procedure on sample reanalysis and the lack of a written distinction between reanalysis and reinjection. This was addressed in the company CAPAs.

Data sets for amodiaquine were found to be saved in a different areas as that described to be those of the study. There were folders entitled "120130",...in sequential order until "120228", however these were verified to be test runs performed using standards or blank, not sample runs.

Storage

The records for the Deep freezer No. CLAB-FR-01 were reviewed and reconciliation verified relative to the number of samples withdrawn and returned and the dates of the injections performed.

In-process audits

There was no procedure to specifically describe at which step in process audits were done. However, in process checks were documented by printing the sequence prior to the start of the injections and by sign off by analyst. There was no QA sign off or supervisor sign off as a 2nd analyst was used as the substitute.

Equipment

There was no separate register for columns. As they were dedicated to each individual equipment and the use of HPLC MS/MS as a whole was recorded, this was considered acceptable.

Columns were stored in bulk in drawers. There was no means of protecting them from shocks as the drawers were opened and closed. This could potentially reduce their effective lifetime of columns.

Pipettes: calibration records were requested for successive time intervals covering the study for pipettes CLAB-P2P-052 and -047. Volumes of 10 to 100 µL and 200 to 1000 µL were covered by the calibration results which were obtained from 10 successive weightings and supported by balance printouts. Acceptance criteria were specified in the associated procedure. The documentation supporting the pipettes was considered to be acceptable overall.

For ultra-deep freezer qualification, temperature mapping was not performed as only 2 probes were used, their placement was not documented in the report other than top and bottom, the regular probe being in the middle. Other studies, such as a study of

the amount of time necessary from shut down to warming to room temperature were done.

Computer access rights

Computer user privileges and access rights were defined specifically for the study. There were 3 persons with administrator privileges at the site including 2 analysts. The inability of changing date and time was verified under access of the analyst. This could be done by the administrator however. It was recommended to install an audit trail for changes to the date and time as well.

Training of staff and assignment of work in bioanalysis

Training records and job descriptions dated September 2012, were consulted for an analyst at the lab. There were records on the information given to staff regarding the specific analyses which they had to perform. Several trainings on SOPs at the laboratory were recorded as having been given in 2012. Written assessments were performed with no specified frequency but this was seen to be done in several instances in 2011. Practical assessments were also performed on known and unknown samples and the difference relative to another analyst and theoretical value was calculated.

Study specific training on the protocol was recorded.

Training documentation appeared to be relatively complete and satisfactory overall in the area of bioanalysis.

Part 3: Conclusion

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, **Study No. CP1103 (Protocol No. CP1104 v04 for artesunate/AmodiaquineHCl 100 /270 mg tablets)** is considered to have been conducted at an acceptable level of compliance with WHO GCP and GLP at Shanghai Xuhui Hospital Phase I Clinical Research Unit and Bioanalytical laboratory. Shanghai Xuhui Central Hospital, 966 HuaiHaiZhong Rd, Shanghai China 200031.

All the non-compliances observed during the inspection that were listed in the full 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 3 years, provided that the outcome of any inspection conducted during this period is positive.