# **Study Plan Nr. CLIXXXX/1E**

**Residue Depletion study after intramuscular administration of AMOXICILLIN in PIGS**

1. **Sponsor and facilities**

**Sponsor**

**Test Facility**

## *Main Facility*

**Clinobs, S.L.**

c/ JosepTarradellas, 2 baixos 1

17820 – Banyoles

Girona (Spain)

Tel: +34 972 583 366

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## *Animal housing and experimental work*

### Granja Experimental Clinobs Cal Fuster

Pujals dels Cavallers, 17844 Cornellà de Terri

Girona (Spain)

#### Quality Assurance Unit

**QA Solutions, S.L.**

Plaça Frederica Montseny 1, 3º 2ª

08203 Sabadell

Barcelona (Spain)

Tel: +34 937 279 043

**Test Site**

Laboratorio 2

1. **Personnel and signatures**

**Sponsor**

|  |  |  |  |
| --- | --- | --- | --- |
| **Monitor** |  |  |  |
|  |  |  |  |
|  | Signature |  | Date |

**Test Facility**

 **Clinobs, S.L.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study Director** |  |  |  |
| Óscar Ciervo*Biologist*ociervo@clinobs.com |  |  |  |
|  | Signature |  | Date |

|  |  |  |  |
| --- | --- | --- | --- |
| **Test Facility Management** |  |  |  |
| Feli Contreras*Veterinary surgeon*fcontreras@clinobs.com |  |  |  |
|  | Signature |  | Date |

 **QA Solutions, S.L.**

|  |  |  |
| --- | --- | --- |
| **Lead Assurance Unit** |  |  |
| Toni Bermúdez*Biologist, PhD*t.bermudez@qasolutionssl.com |  |  |  |
|  | Signature |  | Date |

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1. **Details of the study**
	1. **Study title**

Residue Depletion study after intramuscular administration of AMOXICILLIN in pigs

* 1. **Test item**

AMOXICILLIN

* 1. **Introduction**

Amoxicillin is a beta-lactam antimicrobial derivative of the ampicillin belonging to the group of the aminopenicillins, included in the subgroup of penicillins of wide spectrum sensitive to the beta-lactamases.

All the beta-lactams share the same mechanism of action blocking the synthesis of the wall of the Gram-negative and Gram-positive bacteria, across an inhibition of the transpeptidase. It drives to a prolapse of the cytoplasmatic membrane which affects the formation of spheroplasts and immediately afterwards, the lysis and death of the bacterial cell take place [[[1]](#endnote-1)].

|  |  |
| --- | --- |
| mfcd00056860 | Amoxicillin structure |

The Commite for Veterinary Medicinal Products (CVMP) of the European Agency for the Evaluation of Medicinal Products (EMEA) has established Maximum Residue Levels (MRLs) for Amoxicillin in porcine tissues.

Amoxicillin is included in the Annex 1 of Council Regulation of the European Community no. 2377/90 [**[[2]](#endnote-2)**] with the following MRLs for porcine tissues:

|  |  |  |  |
| --- | --- | --- | --- |
| **Active Subtance** | **Marker Residue** | **Target tissue** | **MRL** |
| Amoxicillin | Amoxicillin | Muscle (including injection site)FatKidneyLiver | 50 μg/kg50 μg/kg50 μg/kg50 μg/kg |

* 1. **Justification and objectives of the Study**

The aim of this Study is to determine the withdrawal period of amoxicillin in pigs treated intramuscularly with AMOXICILLIN.

The pig has been chosen as the target animal for this Study because it is one of the species of destination for the test item.

The intramuscular route is chosen because it is the one by which the active principle is rapidly liberated and reaches maximum concentration in a little time. Moreover, the intramuscular route provides more lasting systemic concentrations than intravenous route.

The prescribed dose regimen will be an intramuscular administration at the dose of XX mg of Amoxicillin /kg b.w.

* 1. **Quality of the Study**

This is a Multi-site Study which will be performed in compliance with the principles of Good Laboratory Practice (GLP), specified in the Royal Decree 1369/2000 of 19 July and based on the OECD principles of Good Laboratory Practice.

The specifications realized about the principles of GLP in the Multi-site Studies by the “Agencia Española de Medicamentos y Productos Sanitarios” and the OECD have been taken in consideration for the correct development of the Study.

The Study will be carried out according to the Standard Operating Procedures (SOPs) of Clinobs, S.L. and in delegated phase to LABORATORIO 2 with their own SOPs.

* 1. **Regulatory Guidelines**

The Study Plan is based on the following guidelines:

* EMEA/CVMP/036/95-FINAL Approach towards harmonisation of withdrawal periods [[[3]](#endnote-3)]
* EMEA/CVMP/542/03 Guideline on Injection Site Residues [[[4]](#endnote-4)]
* EMEA/CVMP/209865/2004 Overview of comments received on Draft Guideline on Injection Site Residues (EMEA/CVMP/542/03-FINAL) [[[5]](#endnote-5)]

The software WT 1.4 used to calculate the withdrawal period is obtained from the following guideline:

* EMEA/CVMP/563/02 Note for guidance on Approach towards harmonisation of withdrawal periods – Updated application software [[[6]](#endnote-6)]

The bioanalytical procedure will be validated in accordance with the following guideline:

* Notice to Applicants (Volume 8) [**[[7]](#endnote-7)**]

The Study will be conducted in compliance with the following Animal Welfare regulations:

* Spanish Royal Decree 1201/2005, 10 October, regarding to the protection of animals used for experimental and other scientific purposes [**[[8]](#endnote-8)**].
* Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [**[[9]](#endnote-9)**].
* Draft Appendix A Of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No.123) [**[[10]](#endnote-10)**]
1. **Test item**
	1. **Description**

The Sponsor is responsible of the identity, batch, composition and concentration of the test item.

The stability of the test item will be defined and conditioned by the specifications sent by the Sponsor.

Any other relevant information like safety precautions must be delivered to the Study Director in order to assure the correct handling of the test item.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Identification: | AMOXICILLIN |  |
|  | Register No. |  |  |
|  | Active principles: |  |  |
|  | Batch: |  |  |
|  | Expiry: |  |  |
|  | Use: | Antibiotic |  |
|  | Storage conditions: | No special conditions |  |
|  | Safety precautions: | Specified in the leaflet of the test item |  |

Once the Study is completed, the remaining test item will be returned back to the Sponsor or eliminated.

A sample of the test item will be kept in the Clinobs, S.L. archives for 5 years from the date of the issue of the Final Report or until its expiry date.

* 1. **Composition**

The composition of the test item AMOXICILLIN is the following:

Amoxicillin 150 mg

Excipients (qs) 1 mL

1. **Experimental System**
	1. **Description**

Clinically healthy pigs with the following characteristics will be used in this study:

|  |  |
| --- | --- |
| Number: | 18 |
| Species: | Pig (*Sus domesticus*) |
| Breed: | Landrace - Large White |
| Origin: | Ramaderia Ayats, S.C.P. (Sant Julià de Ramis, Girona, Spain) |
| Gender: | 9 males and 9 females  |
| Age: | Between 4 and 5 months |
| Initial body weight: |  45 – 55 kg |

As indicated in the medical history of the animals, these animals may not have been treated neither with amoxicillin nor other medication in the previous 30 days.

The animals will undergo an acclimatization period of at least two weeks before the start of the Study under the same conditions as they will be kept during the Study.

* 1. **Justification of the species**

The investigational item is intended to be used in pigs.

* 1. **Identification of the animals**

The animals will be identified with a plastic ear-tag.

* 1. **Housing and environmental conditions**
		1. **Housing**

During all the Study, the animals will be housed individually.

The boxes will be identified with the Study number, Study Director, group of sacrifice, number of animals, sex, animal’s number and experimental starting and ending dates.

* + 1. **Environmental conditions**

The temperature and the relative humidity in the facilities will be controlled, and recorded by means of the Software Comsoft 3.4 (Testo, Barcelona, Spain).

* 1. **Feed and water**
		1. **Feeding regimen**

Each animal will have free access to feed (ad libitum). The feed will be not medicated.

The feed will be manufactured and supplied by Nutrex Banyoles, S.A.

The feed disposed to the animals will be the same during the acclimatization and administration periods. The composition of the feed will be suitable for the development of the animals.

A certificate of analysis will be included in the Final Report.

* + 1. **Watering regimen**

The animals will have free access to non-medicated drinking water provided by Aigües de Banyoles during all the study.

A certificate of analysis will be included in the Final Report.

1. **Experimental design**
	1. **Veterinary inspection**

Before the Study begins, a veterinary examination of the animals will be carried out by a veterinary surgeon. The analysis includes an examination of the animal to evaluate its health condition and the results will be recorded in the clinical veterinary inspection form.

**Clinical parameters to be observed**

Complete physical examination

Rectal temperature

Physical condition: general behaviour

**General physical exploration**

Appearance of skin

Head: nostrils, external ears

General respiratory system

Digestive system: appearance of faeces

Locomotor system: limbs

Genitourinary system: genital organs

* 1. **Animal welfare**

The welfare of the animals will be monitored during the Study. The animals will be examined daily by the technical staff.

If during the acclimatisation period or in the course of the Study the animal is noted to clearly lose weight (more than 10%), if it does not eat well or if its behaviour is not normal it will also be examined by a veterinary surgeon. Any observed clinical signs will be reported on the Clinical Observations Form used by the Testing Facility.

* 1. **Necropsies**

A full necropsy will be carried out by the veterinary surgeon on any animal that dies or must be euthanised for ethical reasons during the acclimatization period or during the Study. Samples of the target organs will be taken and analysed by a veterinary surgeon.

* 1. **Procedures for removal of subjects from the study**

The decision to remove an animal from the Study will be taken by the Study Director after consultation with the veterinary surgeon. The latter will investigate the animal thoroughly and will prepare a detailed report, including a clear rationale for removal.

Blood samples will always be collected ante mortem and analyzed when an animal is to be removed from the experimental part of the Study.

Whenever possible, the Study Director will contact the Study Monitor before any actions/discussions are taken and all actions will be documented and reported.

* 1. **Fate of removed study animals**

Animals removed from the Study will undergo euthanasia to prevent further suffering and will be fully necropsied. During necropsy, if it would be necessary, tissue samples for histopathology will be collected from all organs showing macroscopic pathology and the target ones and will be investigated microscopically for histopathological analysis by a certified facility that will be indicated in a Study Plan Amendment.

All actions will be documented on the appropriate forms, justified and communicated to the Sponsor.

* 1. **Concomitant medication and therapies**

No other treatment than that indicated in this Study Plan should be administered during the course of this Study (including the acclimatization period). If any additional treatment or therapy is considered essential by the veterinary surgeon (e.g. for animal welfare reasons), the type of medication, treatment duration and reason for use will be recorded and the Sponsor will be informed immediately.

If possible, the Study Monitor should be contacted before starting any concomitant treatment.

* 1. **Laboratory analyses**

Before the Study begins, blood biochemistry and haematological analyses will be carried out on all the animals. The blood samples will be obtained from jugular vein. They will be obtained without a prior fasting.

The samples will be delivered refrigerated to LABORATORIO 2 on the same day.

The Principal Investigator for this delegated phase will be xxxxxxxxxxxxxxxxxx.

The phase number of this delegated phase will be XXX.

.

* + 1. **Specimen and samples to test**

18 Serum and blood with EDTA samples will be analyzed as described in this Study Plan.

* + 1. **Samples collection**

LABORATORIO 2 will provide Clinobs, S.L. with the tubes needed for each determination.

* + 1. **Samples identification**

LABORATORIO 2 will provide to Clinobs, S.L. labels for the sample’s identification.

Each animal will be assigned a number that is accordingly specified in the form relevant for this study, and the sample tubes (blood with EDTA and serum) will be labelled with the same number in order to duly identify every animal.

The information of each sample will be introduced in the computer system of LABORATORIO 2

* + 1. **Samples transport and reception**

Study samples (serum and blood with EDTA) will be sent refrigerated to LABORATORIO 2 on the same day of obtention.

Should the samples be received in inadequate or bad conditions, the receiver will have to annotate it in the workbook and will have to report as well the incident to the Study Director by phone or fax.

* + 1. **Analytical determinations**

During the assay, the following determinations will be carried out:

**Haematology analyses**

Total leukocyte count (103/µL)

Differential leukocyte count (%)

Haematocrit (%)

Haemoglobin (g/100 mL)

Mean corpuscular volume: MCV (fL)

Mean corpuscular haemoglobin: MCH (pg)

Mean corpuscular haemoglobin concentration: MCHC (g/100 mL)

Platelet count (103/µL)

Erythrocyte count (106/µL)

**Serum biochemistry**

Glucose (mg/100mL)

Urea (mg/100mL)

Creatinine (mg/100mL)

Glutamic-oxaloacetic transaminase (GOT) (U/L)

Glutamic-pyruvic transaminase (GPT) (U/L)

Alkaline phosphatase (U/L)

Creatinine phospokinase (U/L)

Total protein (g/100mL)

Proteinogram

* + 1. **Material, equipment and methods**

Serum biochemistry

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Method** | **Equipment** | **Supplier** |
| Urea | SpectrophotometryUrease – GLDH – 340 nm | Olympus AU5400 | Olympus |
| Glucose | SpectrophotometryHexokinase method | Olympus AU5400 | Olympus |
| Creatinine | SpectrophotometryJaffé method | Olympus AU5400 | Olympus |
| GOT | SpectrophotometryIFCC method | Olympus AU5400 | Olympus |
| GPT | SpectrophotometryIFCC method | Olympus AU5400 | Olympus |
| Total Protein | SpectrophotometryBiuret method | Olympus AU5400 | Olympus |
| Creatinine phosphokinase | SpectrophotometryIFCC method | Olympus AU5400 | Olympus |
| Alkaline phosphatase | SpectrophotometryIFCC method | Olympus AU5400 | Olympus |
| Proteinogram | Capillary Electrophoresis | CZE-2000 Paragon | IZASA(Beckman) |

Haematology

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Method** | **Equipment** | **Supplier** |
| Total leukocyte count | Impedance |  Pentra DX 120 | ABX-HORIBA |
| Erythrocyte count | Impedance | Pentra DX 120 | ABX-HORIBA |
| Platelet count | Impedance | Pentra DX 120 | ABX-HORIBA |
| Haematocrit | Impedance | Pentra DX 120 | ABX-HORIBA |
| Haemoglobin | Photometry(550 nm Absorbance) | Pentra DX 120 | ABX-HORIBA |

The Differential leukocyte count will be performed manually (according to an internal LABORATORIO 2 SOP) by counting the different leukocytes up to a total of 100 cells. The result will be expressed in percentage. Absolute values will be calculated by applying the obtained percentage to the total leukocyte count.

* + 1. **Quality Controls**

**Internal Quality Controls**

Serum biochemistry:

Control serum 1 of Olympus (Reference ODC0003)

Control serum 2 of Olympus (Reference ODC0004)

Proteinogram:

Normal control of Beckman (Reference 667600)

Abnormal control of Beckman (Reference 667490)

Haematology:

ABX Difftrol N Control (Reference 2062016)

ABX Difftrol L Control (Reference 2062016)

ABX Difftrol H Control (Reference 2062016)

**External Quality Controls**

**FPCQLC** (FUNDACIÓ PEL CONTROL DE QUALITAT DELS LABORATORIS CLÍNICS) Generalitat de Catalunya.

**SEQC** (SOCIEDAD ESPAÑOLA DE QUÍMICA CLINICA)

* 1. **Inclusion and exclusion criteria**

The animals will be assessed and included in the Study in accordance with the following veterinary criteria:

**Inclusion criteria**

Results of analytical profile (clinical analyses) within normality [[[11]](#endnote-11),[[12]](#endnote-12),[[13]](#endnote-13),[[14]](#endnote-14)]

Clinical examination (performed by a veterinary surgeon) within normality

**Exclusion criteria**

General alterations in aspect and colour of skin

Severe respiratory alterations

Alterations in external mucosae

Rectal temperature not normal for this species

Analytical profile (clinical analyses) not within normality [**12**,**13**,**14**,**15**]

Body-weight loss during acclimatization (more than 10% of its body weight)

Animal treated with active principle or with any other medication in the 30 previous days before the Study begins

* 1. **Dose and route of administration**
		1. **Dose**

The prescribed dose regimen will be an intramuscular administration at the dose of XX mg of Amoxicillin /kg b.w.

* + 1. **Route of administration**

The test item will be administered intramuscularly alternatively in both sides of the neck.

The animals will be administered without a prior fasting.

* 1. **Experimental work**

The Study will be carried out with 18 pigs (9 males and 9 females). Sixteen animals will be administered while the remaining animals will form the Control group and will not be administered.

* + 1. **Distribution of the groups**

The animals will be distributed in four groups of four animals each (two males and two females) and a Control group of two animals.

Each group will have undergone a different post-treatment period as follows:

* X days: four animals
* X days: four animals
* X days: four animals
* X days: four animals
	+ 1. **Sacrifice and sample extraction**

The animals will be sacrificed under pentobarbital deep anaesthesia following exsanguination after the post-treatment periods indicated in the Section before.

The Control animals will be sacrificed on the last day of sacrifice.

The tissues to obtain in this Study will be the injection site and surrounding area of the las administration.

A representative sample of each tissue will de obtained from each animal.

|  |  |
| --- | --- |
| **Tissue** | **Sample** |
| Injection site | 500 g ± 20%\* |
| Surrounding area | 300 g ± 20%\* |

\*Size and dimensions will be considered following guideline EMEA/CVMP/542/03 [**5**] and will be duly indicated in the corresponding form

* + 1. **Identification and conservation of the samples**

The samples will be appropriately labelled (code of the Study, animals, group, tissue, date of extraction of the sample) and delivered to LABORATORIO 2 on the same day of the sacrifice.

The samples of injection site and surrounding area will be separately homogenised at Clinobs, S.L. facilities using a mixer before frozen. All samples will be sent frozen to LABORATORIO 2 on the same day of the sacrifice.

All samples will be frozen at –80 ± 10 ºC until their analysis.

For the analysis of amoxicillin levels in injection site and surrounding area, a sample will be cut from the frozen sample without defrosting.

* + 1. **Clinical examination**

All animals will be examined daily by the technical staff to evaluate their health condition and the presence of any adverse symptom.

The clinical signs will be indicated in the corresponding form.

1. **Bioanalytical procedure**

The determination of amoxicillin in injection site and surrounding area from pigs will be carried out according to HPLC-MS/MS methods validated at LABORATORIO 2 (validation code: xxxxxxxxxx).

The complete analytical procedures, including preparation of standard calibration curves and quality control samples, as well as all the bioanalytical data obtained throughout the study will be provided in the Bioanalytical Contributing Report XXXXXXXXX, which will be issued and sent to the Study Director for inclusion in the Final Study Report.

The Principal Investigator for this delegated phase will be XXXXXXXXXXXX.

* 1. **Bioanalytical validation**

The analytical method validations will be performed following Notice to Applicants (Volume 8) [**8**]. The following parameters will be studied:

1. Selectivity:
	* + Analysis of matrix blanks from animal tissues of known origin will be analysed together with a matrix sample spiked separately with the analyte at the limit of quantification (LOQ) and the internal standard.
		+ Acceptance criteria: the area response of the potential interference peaks at the analyte retention time should be lower than 20% of the response of analyte in LOQ. The responses of interference peaks in the internal standard retention time should be ≤ 20% of response of the internal standard at the concentration used in the study (if applicable).
2. Linearity:
	* + The calibration curve will be determined over the concentration range defined (from 0.5x LMR to 8xLMR) using eight calibration standards prepared as matrix samples spiked with known concentrations of analytes. A matrix blank sample and a zero standard sample (blank with internal standard, if applicable) will be prepared and analyzed together with the calibration curve but not used in the calibration function.
		+ The calibration curve will be evaluated in three independent assays.
		+ Acceptance criteria: The correlation coefficient should not be lower than 0.990. At least 66% of the prepared standards should be included in the calibration function, including the LOQ and the highest concentration standard. Outsiders can be discarded.
3. Lower limit of quantification (LLOQ):
	* + Limit of quantification should be one-half the MRL (maximum residue limit) concentration.
		+ It will be determined from 6 matrix samples spiked with the analytes at the lowest concentration of the calibration curve. It will be performed in three independent assays.
		+ Acceptance criteria:
			1. for accuracy, the mean percent deviation of the concentrations of 6 replicates (intra-assay) or 15 replicates (inter-assay) should be within 70-110% of the nominal value
			2. for intra-assay precision, the coefficient of variation of the concentrations of the replicates should be ≤ 20%.
			3. For inter-assay, the coefficient of variation of the concentration of the replicates should be less than the value given by the Horwitz function ().
4. Limit of detection (LOD):
	* + The limit of detection will be determined as 3.3 σ/S where
			1. σ is calculated as the standard deviation of the three values of the y-intercept of the calibration curves in the validation
			2. S is the mean of the slopes of the calibration curves in the validation

* + - Acceptance criteria: the limit of detection must be lower than the LLOQ
1. Precision
	* + Intra-assay: it will be determined in matrix samples (quality control samples) spiked with analytes at three different concentrations: 0.5xLMR, 2xLMR and 5xLMR. Six replicates for each concentration level will be prepared and analyzed in one chromatographic run (18 samples in total).
		+ Inter-assay: the intra-assay experiment will be carried out in three independent assays. A total of eighteen replicates for each concentration level will be prepared (54 samples in total).
		+ Acceptance criteria:
			1. Intra-assay: the coefficient of variation of the concentration of six replicates for each concentration level should be ≤ 15%, except for 0.5xLMR, which can be ≤ 20%
			2. Inter-assay: the coefficient of variation of the concentration of the replicates should be less than the value given by the Horwitz function ()
2. Accuracy:
	* + It will be determined in matrix samples (quality control samples) spiked with analytes at three different concentrations: 0.5xLMR, 2xLMR and 5xLMR). Six replicates for each concentration level will be prepared and analyzed in one chromatographic run (18 samples in total).
		+ Acceptance criteria: the mean percent deviation of the concentrations of the replicates from each concentration level should be within 70-110% of the nominal value
3. Matrix dilution effect:
	* + A matrix sample prepared in sixtuplicate at a concentration higher than the established range (i.e. 40xLMR) will be diluted with control matrix (free of analytes) at a final concentration within the calibration range (i.e. 1/10 dilution). Accuracy and intra precision will be determined.
		+ Acceptance criteria: the same described above for accuracy and intra-assay precision.
4. Stability:
	* + The following stabilities of analytes in matrix (in triplicate at two levels of concentration) will be carried out:
			1. Samples in the autosampler for a period of at least 24 hours.
			2. At short term, matrix samples at room temperature during a time period covering the sample preparation (at least 4 h).
			3. During freeze/thaw cycles (3 cycles).
			4. At long term, during the storage of the frozen samples for a period time to be defined according to the duration of the main study.
		+ Stability of analyte solutions used in the study
	1. **Analysis of study samples**

Samples generated in the biological studies conducted at Clinobs will be analyzed with the validated method using a calibration curve for the quantification of the analytes and quality control samples (prepared in duplicate at three concentration levels) for run acceptance. Samples out of the calibration range will be appropriately diluted with control matrix and re-analyzed. Samples will be analyzed with a single determination.

Calibration curve and quality control samples requirements as well as the acceptance criteria defined for run acceptance are defined as follows:

1. Calibration curve:
	* + The calibration curve will be determined over the concentration range defined in the validation study using eight calibration standards prepared as matrix samples spiked with known concentrations of analytes. A matrix blank sample and a zero standard sample (blank with internal standard, if applicable) will be prepared and analyzed together with the calibration curve but not used in the calibration function.
		+ Acceptance criteria: The correlation coefficient should not be lower than 0.990. At least 75% of the prepared standards should be included in the calibration function, including the LOQ and the highest concentration standard. Outsiders can be discarded.
2. Quality control samples:
	* Quality control samples will be prepared in duplicate in spiked matrix samples at three different analyte concentrations: 0.5xLMR, 2xLMR and 5xLMR.
	* Acceptance criteria: for at least 67% (four of six) of the QC samples, the percent deviation of the concentrations should be within 70-110% of the nominal value. 33% (two of six) of the QC samples may be outside the criteria defined above, but not all at the same concentration.
3. **Evaluation of the results**

The withdrawal time for Amoxicillin in treated pigs will be set in accordance with the criteria laid down in the EMEA Guideline EMEA/CVMP/036/95 of the Committee for Veterinary Medicinal Products [**4**].

The withdrawal time of Amoxicillin for the injection site tissues of the treated pigs will be calculated using the program WT1.4 [**7**] and taking into account the quantified tissue concentrations of the marker residues [**4**].

1. **Summary of the Experimental Work with animals**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Day** | **Body weight** | **Administration** | **Clinical Analyses** | **Clinical Observation** | **Veterinary inspection** | **Obtention of samples** |  |
| Day -14 | **●** |  |  | **●** |  |  |  |
| Day -13 |  |  |  | **●** |  |  |  |
| Day -12 |  |  |  | **●** |  |  |  |
| Day -11 |  |  |  | **●** |  |  |  |
| Day -10 | **●** |  |  | **●** |  |  |  |
| Day -9 |  |  |  | **●** |  |  |  |
| Day -8 |  |  |  | **●** |  |  |  |
| Day -7 |  |  |  | **●** |  |  |  |
| Day -6 | **●** |  | **●** | **●** | **●** |  |  |
| Day -5 |  |  |  | **●** |  |  |  |
| Day -4 |  |  |  | **●** |  |  |  |
| Day -3 | **●** |  |  | **●** |  |  |  |
| Day -2 |  |  |  | **●** |  |  |  |
| Day -1 | **●** |  |  | **●** |  |  |  |
| Day 1  |  | **●** |  | **●** |  |  |  |
| Days 2 to XX |  |  |  | **●** |  | Days XX, XX, XX and XX  |  |
|  |  |  |  |  |  |  |  |

1. **Quality assurance**
	1. **Good Laboratory Practice**

This Study will be carried out according to the principles of Good Laboratory Practice (GLP) specified in Real Decreto (Royal Decree) 1369/2000 of 19 July (Spain) and based on the OECD Principles of Good Laboratory Practice (as revised in 1997), C (97) 186/Final, Paris, 26 November 1997.

The Clinobs, S.L. Quality Assurance Unit will act as Lead Quality Assurance. It will be responsible of verifying that the Study has been carried out complying with the principles of Good Laboratory Practice and that the contribution of the Principal Investigator has been appropriately incorporated in the final Report.

The different phases of the Study done in Clinobs, S.L. will be inspected to determine whether the Study is carried out according to the principles of GLP, the Study Plan of the Study and the Standard Operating Procedures.

The Quality Assurance Unit of LABORATORIO 2 will inspect the works of the phase delegated, also to determine whether the delegated phase of the Study is carried out according to GLP, the Study Plan and the corresponding SOPs.

The report of the phase delegated emitted by the Principal Investigator of LABORATORIO 2 will be audited by their own Quality Assurance to confirm that the methods, procedures and observations reflect faithfully the raw data of the Study.

The Head of the Quality Assurance Unit of LABORATORIO 2 will prepare and sign a statement including types of inspections and their dates, including the phase(s) of the Study inspected.

The Final Report will be audited by the Lead Quality Assurance to confirm that the methods, procedures and observations reflect faithfully the raw data of the Study.

The Head of the Lead Quality Assurance Unit will prepare and sign a statement including types of inspections and their dates, including the phase(s) of the Study inspected.

* 1. **Amendments and Deviations to the Study Plan**

Amendments (planned changes) to the Study Plan after the Study initiation date will be issued and signed by the Study Director and the Study Monitor. The amendments will be distributed and added to all the copies of the Study Plan.

Deviations (unplanned changes) to the Study Plan will be described, justified, acknowledged and dated by the Study Director and/or Principal Investigator, and maintained with the Study raw data. The Sponsor will be promptly informed of any relevant deviations from the Study Plan, and the Final Report will reflect all these relevant deviations.

1. **Report**

Once the Study has been finalized, the Study Director will elaborate a Final Report. This Final Report will content at least all relevant information regarded to the Study, as detailed below:

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  |  |
|  | Identification of the Study | * A descriptive title.
* Study identification.
* Identification of the test item by code or name.
* Characterisation of the test item including purity, stability and homogeneity.
 |  |
|  | Sponsor and test facilities | * Name and address of the sponsor.
* Name and address of the test facilities and test sites involved.
* Name and address of the Study Director.
* Name and address of the Principal Investigator(s) and the phase(s) of the study delegated, if applicable.
* Name and address of scientists having contributed reports to the final report.
 |  |
|  | Dates | * Start and end of the experimental work.
 |  |
|  | Study Director / Principal Investigator statement  | * Accepting the validity of the data and indicating the extent to which the study complies with Principles of GLP.
 |  |
|  | QA Statement | * Type of inspections and dates.
* Phase(s) of the Study inspected.
 |  |
|  | Description  | * Description of methods and materials used.
* Reference to test guideline or method.
 |  |
|  | Results | * A summary of the results.
* All information and data required by the Study Plan.
* A presentation of the results, including a statistical analysis.
* An evaluation and discussion of the results, as well as a conclusion when necessary.
* Any significant Study Plan deviation.
 |  |
|  | Archive | * Study Plan.
* Any Study Plan amendments and deviations.
* Raw data.
* Final Report.
* Samples of test item.
* When appropriate, samples of the reference item and specimens.
 |  |
|  |  |  |  |

The Final Report will be signed and dated by the Study Director and/or the Principal Investigator and any report included in the Final Report will be signed and dated by the scientist author.

The Principal Investigator will edit a Contributive Report that will be appended in the Final Report. This report will be duly dated and signed.

A draft report will be sent to the Sponsor for evaluation. Taking their comments into account, a QA-audited Final Report will be issued. A copy of the Final Report will be provided.

1. **Archive**

Once the Study has been finalized Clinobs, S.L. will storage the Study Plan, any Study Plan amendments and deviations, raw data, the Final Report, sample of test item(s) and, if it is appropriate, sample of the reference item.

Clinobs, S.L. will keep the documentation for a minimum of five years since the edition of the Final Report. After this period it will be returned to the Sponsor.

Samples of test item will be kept only as long as the quality of the preparation permits their evaluation. No data will be disposed of without the Sponsor’s authorization.

LABORATORIO 2 will keep the documentation for a minimum of 5 years since the edition of the Reports.

Frozen biological samples stored at LABORATORIO 2 will be destroyed three months after issue of the final phase report unless otherwise requested by the Study Director and/or the Study Sponsor.

1. **Schedule of the Study and proposed Study dates**

The following scheme shows a summary of the experimental procedure and the schedule of the Study:

|  |  |
| --- | --- |
| **Draft Study Plan** | Will be specified in a Study Plan amendment |
| **Acclimatization of the animals** | Will be specified in a Study Plan amendment |
| **Experimental work** | Will be specified in a Study Plan amendment |
| **Edition of the contributive Reports** | Will be specified in a Study Plan amendment |
| **Shipment of the Draft Report** | Will be specified in a Study Plan amendment |
| **QAU Audit** | Will be specified in a Study Plan amendment |
| **Final Report** | Will be specified in a Study Plan amendment |

These dates are subjected to a two-week period of delay due to external reasons or by mutual agreement between the Sponsor and the Study Director.

If these dates should change in accordance with the reasons stated in the first Section of the Contract, by mutual agreement between the parties, the definitive dates will be established in a Study Plan Amendment.

1. **Bibliography**
1. [] H.C. Neu (1977) Antimicrobial action and mechanisms of resistance -Vet.Med./Small Animal Clin., Suppl., 683-691. [↑](#endnote-ref-1)
2. [] Consolidated version of the Annexes I to IV of Council Regulation No. 2377/90 updated on 08.04.2006. The European Agency for the Evaluation of Medicinal Products (EMEA), Veterinary Medicines Evaluation Unit, Committee for Veterinary Medicinal Products [↑](#endnote-ref-2)
3. [] EMEA/CVMP/036/95-FINAL Approach towards harmonisation of withdrawal periods [↑](#endnote-ref-3)
4. [] EMEA/CVMP/542/03 Guideline on Injection Site Residues [↑](#endnote-ref-4)
5. [] EMEA(CVMP/209865/2004 Overview of comments received on Draft Guideline on Injection Site Residues (EMEA/CVMP/542/03-FINAL) [↑](#endnote-ref-5)
6. [] EMEA/CVMP/563/02 Note for guidance on Approach towards harmonisation of withdrawal periods – Updated application software [↑](#endnote-ref-6)
7. [] Notice to Applicants and Guideline (Volume 8). Establishment of Maximum Residue Limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin. [↑](#endnote-ref-7)
8. [] Spanish Royal Decret 1201/2005, 10 october, regarding to the protection of animals used for experimental and other scientific purposes. [↑](#endnote-ref-8)
9. [] Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Federation of Animal Sciences Societies. First revised Edition. January 1999. [↑](#endnote-ref-9)
10. [] Draft Appendix A of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No.123). Guidelines for accommodation and care of animals (Article 5 of the Convention) for the animal welfare assessment. [↑](#endnote-ref-10)
11. []Wilfried Kraft and Ulrich M. Dürr. Klinische Labordiagnostik in der Tiermedizin. F.K. Schattauer Verlag GmbH; 4th edition (2000). [↑](#endnote-ref-11)
12. [] Bernard F. Feldman, Joseph G. Zinkl and Nemi C. Jain. Schalm’s Veterinary Hematology. Lippincott Williams & Wilkins; 5th edition (2000). [↑](#endnote-ref-12)
13. [] J. Jerry Kaneko, John W. Harvey and Michael L. Bruss. Clinical Biochemistry of Domestic Animals. Academic Press. 5th edition (1997). [↑](#endnote-ref-13)
14. [] El manual Merck de veterinaria. 5ª edición (2000). [↑](#endnote-ref-14)