Humoral allergenicity testing

Standard protocols

Active cutaneous anaphylaxis (Guinea pig)

Study outline

Experimental animals	Hartley strain guinea pigs
Sex, age at acclimatization	female, 4 weeks
Acclimatization period	1 week
Health status	SPF
Total number of animals	15

Guinea pigs are orally administered with the test substance five times per week via gavage over a period of 4 weeks*. Negative control animals are administered with the vehicle alone at the same volumes and time schedule. Positive control animals are intramuscularly immunized with the test item emulsified in complete Freund's adjuvant (CFA) followed by two further immunisations with the test item emulsified in incomplete Freund's adjuvant (IFA) in two weeks intervals. All animals are rested for one week after the last treatment.

** **Of note:** Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item. This may strongly influence the final price of the study.*

Guinea pigs immunized or otherwise treated with the test item or control item are shaved on their back one day before the test. The test animals are injected intravenously with 0.5 ml Evans blue (2% in physiol. saline) followed by an intradermal challenge with various amounts of the test item in 0.1 ml suspension. At least three 3-fold dilutions of the test item are subcutaneously administered to each test animal as well as to each negative and positive control animal. The animals are sacrificed under anaesthesia 30 min after challenge and diameters of the blue spots, resulting from the inflammatory reaction, are recorded from inside the skin.

Tabulated summary

Group	No. of animals	Sex	Item*	Route*	Frequency* of administration
I	5	F	Test item	oral	20 x (within 4 weeks)
II	5	F	Vehicle	oral	20 x (within 4 weeks)
III	5	F	Test item	i.m. with adjuvant	3 x (within 4 weeks)

* Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item.

Possible modifications

- For exploratory purposes the test battery may be reduced to three animals (or even one animal) per group. In this case, however, the study is unlikely to be accepted by regulatory agencies.
- For more detailed studies the test item may be administered in three dosages (low dose, intermediate dose, high dose). For most regulatory purposes, however, a single dosage is sufficient to meet regulatory requirements.

Time line and Prices

Time from finalization of the study plan to reporting
(first draft report): **8 Weeks**

Price for the study outlined above: **call**

Active systemic anaphylaxis (Guinea pig)

Study outline

Experimental animals	Hartley strain guinea pigs
Sex, age at acclimatization	female, 4 weeks
acclimatization period	1 week
Health status	SPF
Total number of animals	15

Guinea pigs are orally administered with the test substance five times per week via gavage over a period of 4 weeks*. Negative control animals are administered with the vehicle alone at the same volumes and time schedule. Positive control animals are intramuscularly immunized with the test item emulsified in complete Freund's adjuvant (CFA) followed by two further immunisations with the test item emulsified in incomplete Freund's adjuvant (IFA) in two weeks intervals. All animals are rested for one week after the last treatment.

** **Of note:** Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item. This may strongly influence the final price of the study.*

Guinea pigs immunized or otherwise treated with the test item or control item are challenged by injecting them intravenously with 1 ml of the test item at a concentration of 1 mg/ml**. Clinical symptoms of anaphylactic shock are graded as follows:

- no symptoms: **-**
- nose rubbing and/or pile erection: **±**
- weakness, dyspnoea, coughing, sneezing, vomiting and/or wheezing: **+**
- convulsions, cyanoses and/or collapse: **++**
- death: **+++**

*** **Of note:** The challenge dose may be adapted depending on the biological/chemical nature of the test item. This will not influence the final price of the study.*

Tabulated summary

Group	No. of animals	Sex	Item*	Route*	Frequency* of administration
I	5	F	Test item	oral	20 x (within 4 weeks)
II	5	F	Vehicle	oral	20 x (within 4 weeks)
III	5	F	Test item	i.m. with adjuvant	3 x (within 4 weeks)

* Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item.

Possible modifications

- For exploratory purposes the test battery may be reduced to three animals (or even one animal) per group. In this case, however, the study is unlikely to be accepted by regulatory agencies.
- For more detailed studies the test item may be administered in three dosages (low dose, intermediate dose, high dose). For most regulatory purposes, however, a single dosage is sufficient to meet regulatory requirements.

Time line and Prices

Time from finalization of the study plan to reporting
(first draft report): **8 Weeks**

Price for the study outlined above: **call**

Homologous passive cutaneous anaphylaxis (Guinea pig -> Guinea pig)

Protocol A

(used in case the vehicle alone can not be excluded to be sensitising)

Study outline

Experimental animals (donors)	Hartley strain guinea pigs	**Challenge animals (recipients)**	Hartley strain guinea pigs
Sex, age at acclimatization	female, 4 weeks	Sex, age at acclimatization	female, 8 weeks
acclimatization period	1 week	acclimatization period	1 week
Health status	SPF	Health status	SPF
Total number of animals	13	Total number of animals	6

For sensitisation donor Guinea pigs are orally administered with the test substance five times per week via gavage over a period of 4 weeks*. Control animals are administered with the vehicle alone at the same volumes and time schedule. Positive control animals are intramuscularly immunized with the test item emulsified in complete Freund's adjuvant (CFA) followed by two further immunisations with the test item emulsified in incomplete Freund's adjuvant (IFA) in two weeks intervals. All animals are rested for one week after the last treatment. After the resting period blood is taken from all donor animals for the generation of sera. Sera from positive control animals are pooled.

** **Of note:** Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item. This may strongly influence the final price of the study.*

Naive recipient Guinea pigs are passively sensitised by intradermal injection of 0.1 ml of Guinea pig donor antisera serially threefold diluted (1:3:9) with saline according to figure 1. The animals are challenged by intravenous injection of 1 ml of the test item (1mg/animal**) diluted in saline containing 10 mg/ml Evan's blue 24 hours after the intradermal sensitisation. The animals are sacrificed 30 min after the challenge, and the diameters of the blue spots, resulting from the inflammatory reaction, are recorded from inside the skin. A blue spot with a mean diameter of less than 5 mm is graded as negative.

** ***Of note:*** *The challenge dose may be adapted depending on the biological/chemical nature of the test item. This will not influence the final price of the study.*

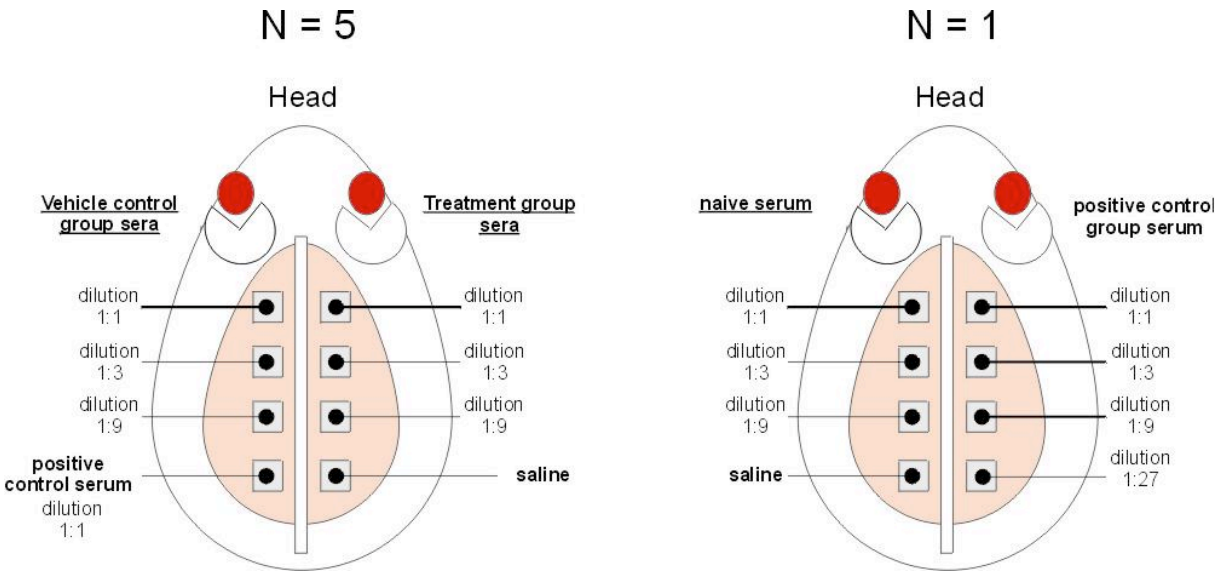

Figure 1: Schematic drawing of localizations of intradermal sensitisation sites of recipient Guinea pigs

Tabulated summary

Donor groups

Group	No. of animals	Sex	Item*	Route*	Frequency* of administration
I	5	F	Test item	oral	20 x (within 4 weeks)
II	5	F	Vehicle	oral	20 x (within 4 weeks)
III	3	F	Test item	i.m. with adjuvant	3 x (within 4 weeks)

* Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item.

Recipient groups

Group	No. of animals	Sex	Sensitisation with sera from		Comment
			Left side	Right side	
IV	5	F	Group II	Group I	sensitisation test
V	1	F	naive serum	Group III	assay control

Possible modifications

- For exploratory purposes the test battery may be reduced to three animals (or even one animal) per group. In this case, however, the study is unlikely to be accepted by regulatory agencies.
- For more detailed studies the test item may be administered in three dosages (low dose, intermediate dose, high dose). For most regulatory purposes, however, a single dosage is sufficient to meet regulatory requirements.

Time line and Prices

Time from finalization of the study plan to reporting
(first draft report): **10 Weeks**

Price for the study outlined above: **call**

Protocol B

(used in case the vehicle alone can definitively be excluded to be sensitising)

Study outline

Experimental animals (donors)	Hartley strain guinea pigs	**Challenge animals (recipients)**	Hartley strain guinea pigs
Sex, age at acclimatization	female, 4 weeks	Sex, age at acclimatization	female, 8 weeks
acclimatization period	1 week	acclimatization period	1 week
Health status	SPF	Health status	SPF
Total number of animals	8	Total number of animals	6

For sensitisation donor Guinea pigs are orally administered with the test substance five times per week via gavage over a period of 4 weeks*. Positive control animals are intramuscularly immunized with the test item emulsified in complete Freund's adjuvant (CFA) followed by two further immunisations with the test item emulsified in incomplete Freund's adjuvant (IFA) in two weeks intervals. All animals are rested for one week after the last treatment. Before the first administration of the test substance (pre-treatment serum), and after the resting period (post-treatment serum) blood is taken from all donor animals for the generation of sera. Sera from positive control animals are pooled.

** **Of note:** Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item. This may strongly influence the final price of the study.*

Naive recipient Guinea pigs are passively sensitised by intradermal injection of 0.1 ml of Guinea pig donor antisera serially threefold diluted (1:3:9) with saline according to figure 2. The animals are challenged by intravenous injection of 1 ml of the test item (1mg/animal**) diluted in saline containing 10 mg/ml Evan's blue 24 hours after the intradermal sensitisation. The animals are sacrificed 30 min after the challenge, and the diameters of the blue spots, resulting from the inflammatory reaction, are recorded from inside the skin. A blue spot with a mean diameter of less than 5 mm is graded as negative.

*** **Of note:** The challenge dose may be adapted depending on the biological/chemical nature of the test item. This will not influence the final price of the study.*

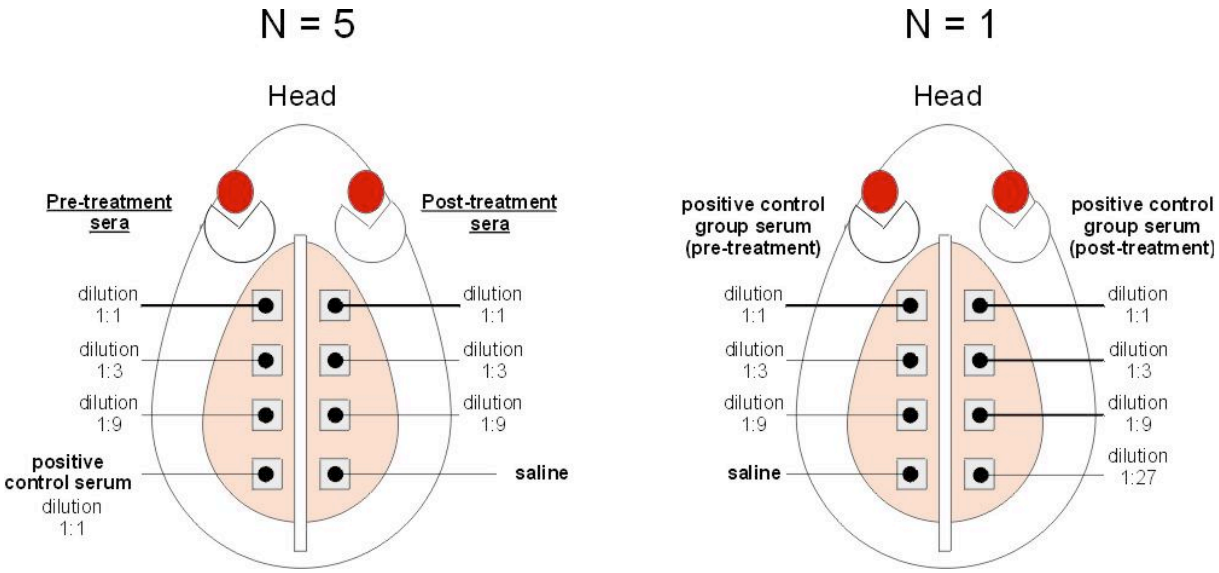

Figure 2: Schematic drawing of localizations of intradermal sensitisation sites of recipient Guinea pigs

Tabulated summary

Donor groups

Group	No. of animals	Sex	Item*	Route*	Frequency* of administration
I	5	F	Test item	oral	20 x (within 4 weeks)
II	3	F	Test item	i.m. with adjuvant	3 x (within 4 weeks)

* Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item.

Recipient groups

Group	No. of animals	Sex	Sensitisation with sera from		Comment
			Left side	Right side	
IV	5	F	Group I (pre-treatment)	Group I (post-treatment)	sensitisation test
V	1	F	Group II (pre-treatment)	Group II (post-treatment)	assay control

Possible modifications

- For exploratory purposes the test battery may be reduced to three animals (or even one animal) per group. In this case, however, the study is unlikely to be accepted by regulatory agencies.
- For more detailed studies the test item may be administered in three dosages (low dose, intermediate dose, high dose). For most regulatory purposes, however, a single dosage is sufficient to meet regulatory requirements.

Time line and Prices

Time from finalization of the study plan to reporting
(first draft report): **10 Weeks**

Price for the study outlined above: **call**

Combined testing package	
Active systemic and passive cutaneous anaphylaxis	**call**

Heterologous passive cutaneous anaphylaxis (mouse -> rat)

Study outline

Experimental animals (donors)	BALB/c mice	**Challenge animals (recipients)**	Wistar rats
Sex, age at acclimatization	female, 4 weeks	Sex, age at acclimatization	female, 8 - 12 weeks
acclimatization period	1 week	acclimatization period	1 week
Health status	SPF	Health status	SPF
Total number of animals	15	Total number of animals	11

For sensitisation BALB/c mice are orally administered with the test substance five times per week via gavage over a period of 4 weeks*. Control animals are administered with the vehicle alone at the same volumes and time schedule. Positive control mice are intraperitoneally immunized with the test item emulsified in complete Freund's adjuvant (CFA) followed by two further intraperitoneal immunisations with the test item emulsified in incomplete Freund's adjuvant (IFA) in two weeks intervals. All animals are rested for one week after the last treatment. After the resting period blood is taken from the retroorbital plexus of all mice for the generation of sera. Sera from positive control animals are pooled.

* ***Of note:*** *Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item. This may strongly influence the final price of the study.*

Naive Wistar rats are passively sensitised by intradermal injection of 0.1 ml of mouse antisera serially threefold diluted (1:3:9) with saline according to figure 3. The animals are challenged by intravenous injection of 1 ml of the test item (1mg/animal**) diluted in saline containing 10 mg/ml Evan's blue 24 hours after the intradermal sensitisation. The animals are sacrificed 30 min after the challenge, and the diameters of the blue spots, resulting from the inflammatory reaction, are recorded from inside the skin. A blue spot with a mean diameter of less than 5 mm is graded as negative.

** ***Of note:*** *The challenge dose may be adapted depending on the biological/chemical nature of the test item. This will not influence the final price of the study.*

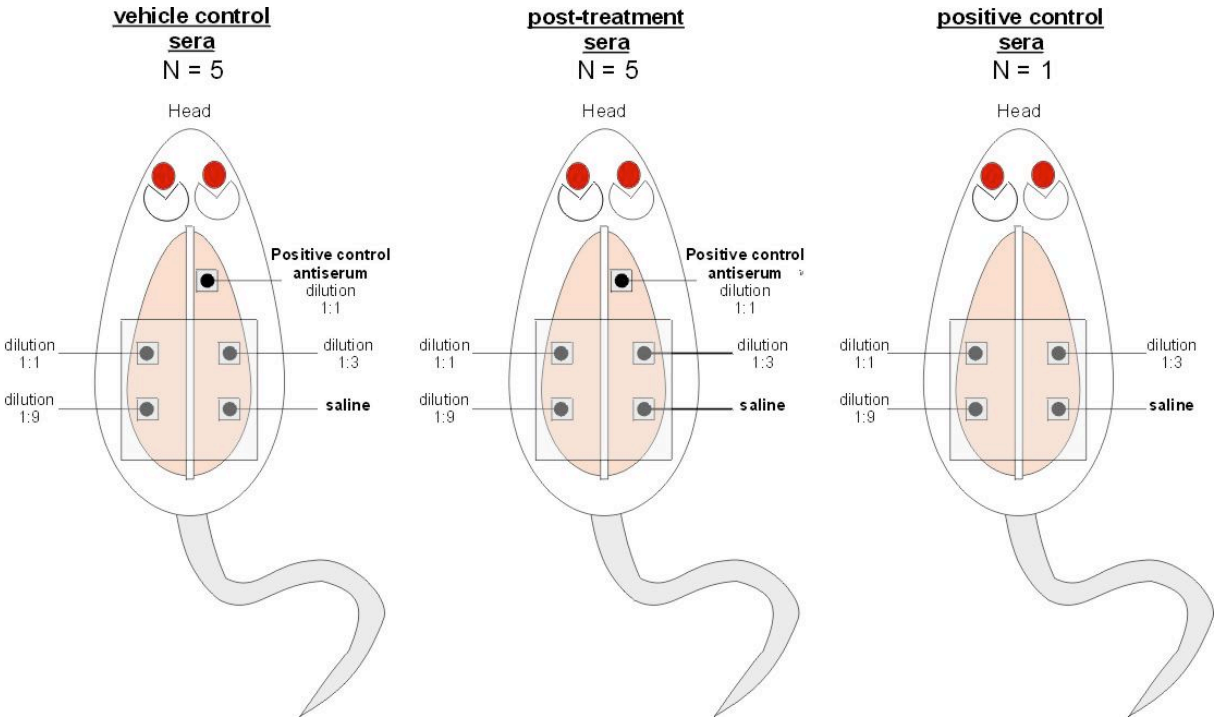

Figure 3: Schematic drawing of localizations of intradermal sensitisation sites of recipient rats

Tabulated summary

Donor groups

Group	No. of animals	Sex	Item*	Route*	Frequency* of administration
I	5	F	Test item	oral	20 x (within 4 weeks)
II	5	F	Vehicle	oral	20 x (within 4 weeks)
III	5	F	Test item	i.m. with adjuvant	3 x (within 4 weeks)

* Routes of administration, frequencies of administration, and duration of the treatment may be adapted to reflect the intended clinical use of the test item.

Recipient groups

Group	No. of animals	Sex	Sensitisation with sera from	Comment
IV	5	F	Group II	sensitisation test (vehicle)
V	5	F	Group I	sensitisation test (test item)
VI	1	F	Group III	assay control

Possible modifications

- For exploratory purposes the test battery may be reduced to three animals (or even one animal) per group. In this case, however, the study is unlikely to be accepted by regulatory agencies.
- For more detailed studies the test item may be administered in three dosages (low dose, intermediate dose, high dose). For most regulatory purposes, however, a single dosage is sufficient to meet regulatory requirements.

Time line and Prices

Time from finalization of the study plan to reporting
(first draft report): **10 Weeks**

Price for the study outlined above: **call**