

RESPIRATORY RGT

INSTRUCTION MANUAL Respiratory Gene Technology

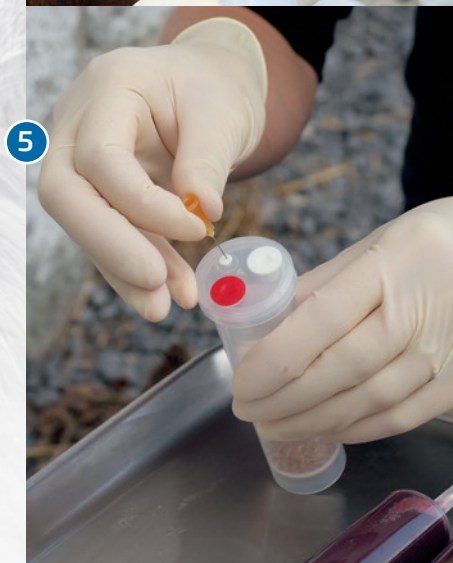
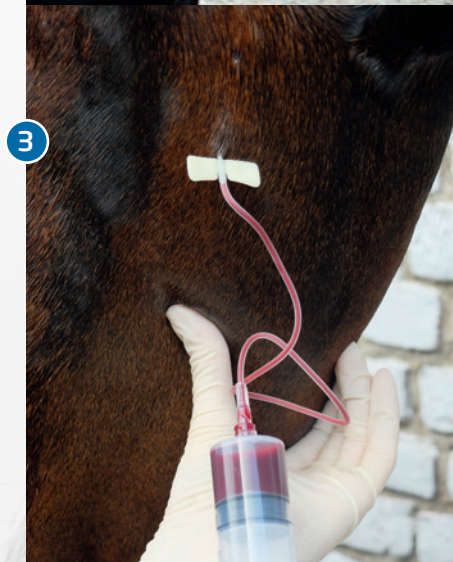
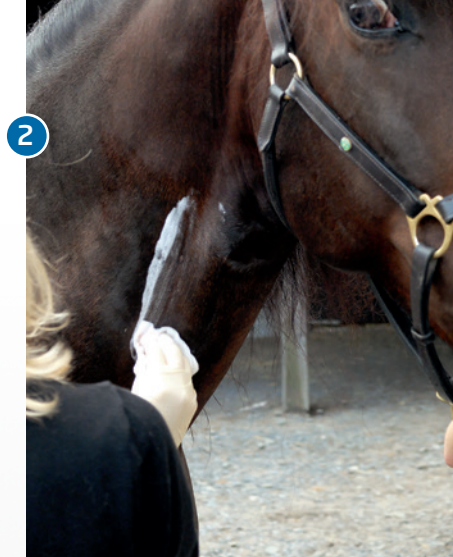




Box 1 Stable instruction

- 1 Make sure the horse was rested for 12 hours prior to blood collection.
- 2 Prep the jugular vein in a sterile fashion.
- 3 Collect 60 ml of venous blood, using the 60 ml syringe and the butterfly cannula.
- 4 Aspirate the blood slowly without too much pressure.
- 5 Place the air-vent 25G needle into the small white plug through the lid of the RGT container.
- 6 Transfer the blood into the RGT container, by placing the 14G needle through the red plug. Inject the blood carefully by holding the needle against the wall of the container.
- 7 Pull the needles out and turn the RGT container gently upside down 10 times.

Repeat these instructions 3 times.





Box 2 Lab instruction

- 1 Place the RGT containers in the incubator at 37°C for 72 hours.
- 2 After incubation, take the RGT containers out, place them in the centrifuge and spin for 10 minutes at 4800 rounds per minute (G-force: 2300).
- 3 Take the containers out of the centrifuge. The brown color and cloudy view of the blood is normal.
- 4 Place the air-vent needle through the small white plug.
- 5 Aspirate from each container 25-30 ml of serum (prevent aspiration of cells) using the 30 ml syringe and the long needle through the large white plug.

* In seldom cases, a fibrin cloth can prevent the collection of serum after centrifugation. In this case it is recommended to stir the content with the long needle and repeat centrifugation.

In the case of an unsatisfactory recovery of serum, stir the blood and repeat centrifugation.



Box 2 Lab instruction

- 6 Place the sterile filter on the 30 ml syringe.
- 7 Place the adapter on the sterile filter.
- 8 Place the 10ml syringe to the adapter.
- 9 Transfer 9-10 ml of serum through the filter into the 10 ml syringe. Change the filter, when it blocks and no more serum passes through. Do not hesitate to apply some pressure during filtration.
- 10 Place a combi-stopper on the syringes.
- 11 Freeze the serum at -20°C.



ADDENDUM

- * Normal recovery from 60 ml full blood is between 25 and 30 ml.
- * Notice that if the horse is on medication at the time of collection, this should be noted in relation to dope-testing.
- * Expect a higher degree of haemolysis after incubation for 72 hours compared to 24 hours. The method of collection and individual variation in the stability of red blood cells from horse to horse will have an influence on the degree of haemolysis in the filtrated serum.

Protocol

Inject the patient twice a week with 9-10 ml of serum intra-muscular.

One RGT kit provides the minimum treatment cure of 4,5 weeks.

Depending on the level and intensity of training and the condition of the horse, treatment can be maintained throughout the season, preferably starting 1-2 weeks in advance. We advise a 6 week treatment of 2 injections/week, followed by 1 injection/week maintenance treatment.

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