# A safer and cleaner trichinella testing method for swine carcases

## by Patrik Bulhozer, Thermo Fisher Scientific.

richinellosis is a zoonotic disease (can be transmitted from animals to humans) that occurs worldwide and is caused by the larvae of the nematode (roundworm), Trichinella. Pigs can become infected through feeding on contaminated animal waste products, exposure to living or dead rodents or other wildlife infected with Trichinella larvae, or cannibalism within an infected herd.

Humans can be infected by eating raw or insufficiently cooked meat from infected animals. While human trichinellosis can be a debilitating disease and may result in death, in animals, the infection is clinically unapparent. Therefore, under the European Commission (EC) Directive No. 2015/1375 (formerly No 2075/2005), all pigs slaughtered for human consumption must be tested for Trichinella spp. by artificial digestion.

# **Historical testing**

Testing methods for the detection of Trichinella infection in wild and domesticated animals has historically been achieved by either:

 Directly demonstrating the parasite's presence in tissue samples; or
 Indirectly demonstrating the parasite's presence by using immunological methods to detect specific

### Fig. 1. Digestion residue.



antibodies to Trichinella spp. in blood, serum or tissue fluid samples.

The artificial digestion method is based on the use of pepsin and has several drawbacks, including sourcing and supply of sufficient amounts of pepsin, quality of pepsin, and lack of standardisation of materials that often requires retesting.

Plus, any time workers handle pepsin or hydrochloric acid during the artificial digestion process, they are at risk of serious injury.

# New and safer solution

PrioCHECK Trichinella AAD from Thermo Fisher Scientific offers abattoirs and meat packers a cleaner, safer way to test swine carcases for potentially dangerous Trichinella at meat inspection. The alternative artificial digestion (AAD) method has been validated and approved by the European Union and is now listed in commission regulation (EC) 2015/1375.

PrioCHECK Trichinella AAD uses a serin-endopeptidase, part of the enzyme group of subtilisins. It is a reliable testing alternative to the currently used pepsin-based artificial digestion method because it uses a recombinant enzyme from a standardised, secure production facility, ensuring good availability of the enzyme and consistent quality.

In addition, it does not use pepsin powder or hydrochloric acid, increasing ease of handling and worker safety.

# **Testing methodology**

Laboratories converting to this innovative new method do not have to change their testing routine since the protocol of the sample preparation method follows the same steps as that of the currently used pepsinbased method. A piece of muscle tissue is chopped, minced and then digested with the digestive enzyme subtilisin. The assay runs at a slightly basic pH. The digestion solution is filtered, and after sedimentation steps, it is examined under microscope for the presence of the larvae.



#### PrioCHECK Trichinella AAD Procedure

A. Digest chopped meat in enzyme solution for 20 minutes. B. Pour digestion solution through a sieve into a separate funnel and let the sample sediment. Run off 75ml digestion solution into a tube. C. Let the digestion solution sediment and discard 65ml of the supernatant. D. The remaining 10ml is examined for the presence of Trichinella larvae in a petri dish.

All components of the PrioCHECK Trichinella AAD are liquid solutions, and no acid is added. Therefore, the risk associated with handling powder or concentrated acids are avoided.

# Procedure

• Heat up the digestion buffer and enzyme solution provided with the kit to 60°C.

• Add chopped meat and incubate for 20 minutes.

• Pour digestion solution through a sieve into a separation funnel.

 Let the digestion solution stand for 30 minutes to allow sedimenta-

tion. • Transfer 75mL digestion solution into a tube.

• Let the digestion solution stand for 10 minutes to allow for separation

Discard 65mL of the supernatant.
Optional wash step: if high

amounts of debris remain, add 30mL water, mix, allow solution to stand for 10 minutes to allow for separation and then discard 30mL of supernatant.

• Pour the remaining I 0mL into a petri dish and examine for the presence of Trichinella larvae.

# Validated by the CRL

The Community Reference Laboratory (CRL) for Trichinella in Rome has validated the performance of the PrioCHECK Trichinella AAD Kit and has approved the product as an official method for use in the in vitro detection of Trichinella spp. in meat from domestic swine. The performance of the PrioCHECK Trichinella AAD has also been evaluated in more than 100 digestion runs.

The samples, obtained from the European Reference Laboratory for Parasites (EURLP) and Nationales Referenzlabor für Trichinella, BfR in Germany, included samples from abattoirs, experimentally infected animals, and spiked samples.

Results show that the remaining residue on the sieve was below 5% of the starting meat tissue, which is fully compliant with the related guideline requirements.

#### Conclusion

PrioCHECK Trichinella AAD is easier to handle than the pepsinbased method, resulting in a safer work environment.

This is by eliminating the need for pepsin and acids, and also offers high quality standards with certified production through a quality management system.

Monitoring health at slaughter is an essential way to prevent the transfer of animal disease to humans and helps ensure the safety of our food source.