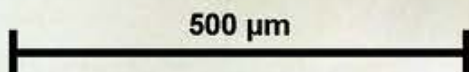




# Cloudy Digestion Fluid: A Challenge in Trichinella Detection Using the Artificial Digestion Method

**Behdad Tarbiat,**

Swedish Veterinary Agency



# Our little problem





*No One Knows*

# Our little problem



Cause      Effect

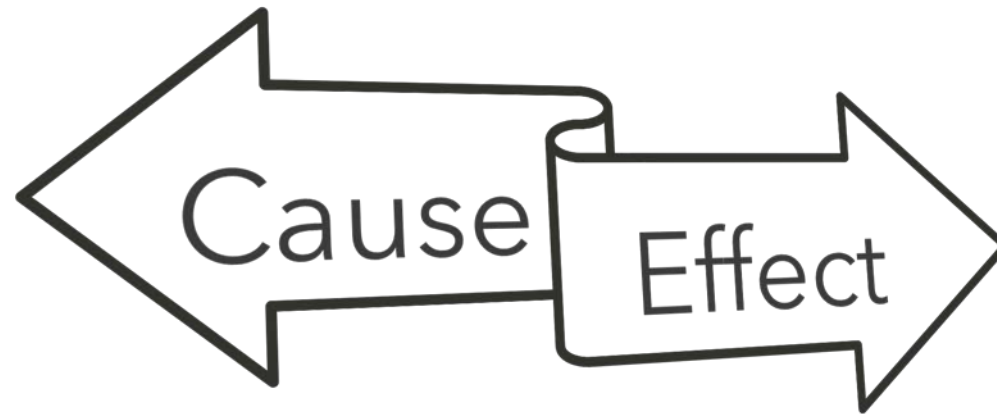
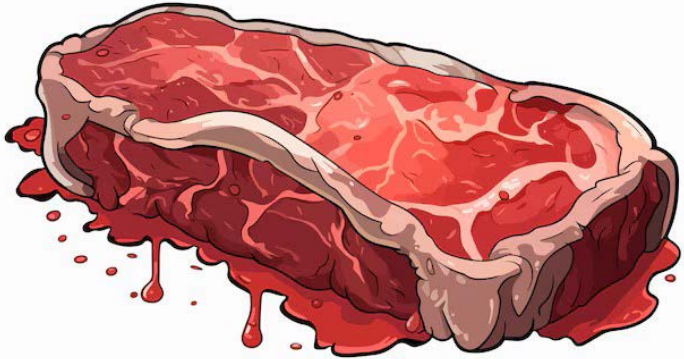


# Our little problem



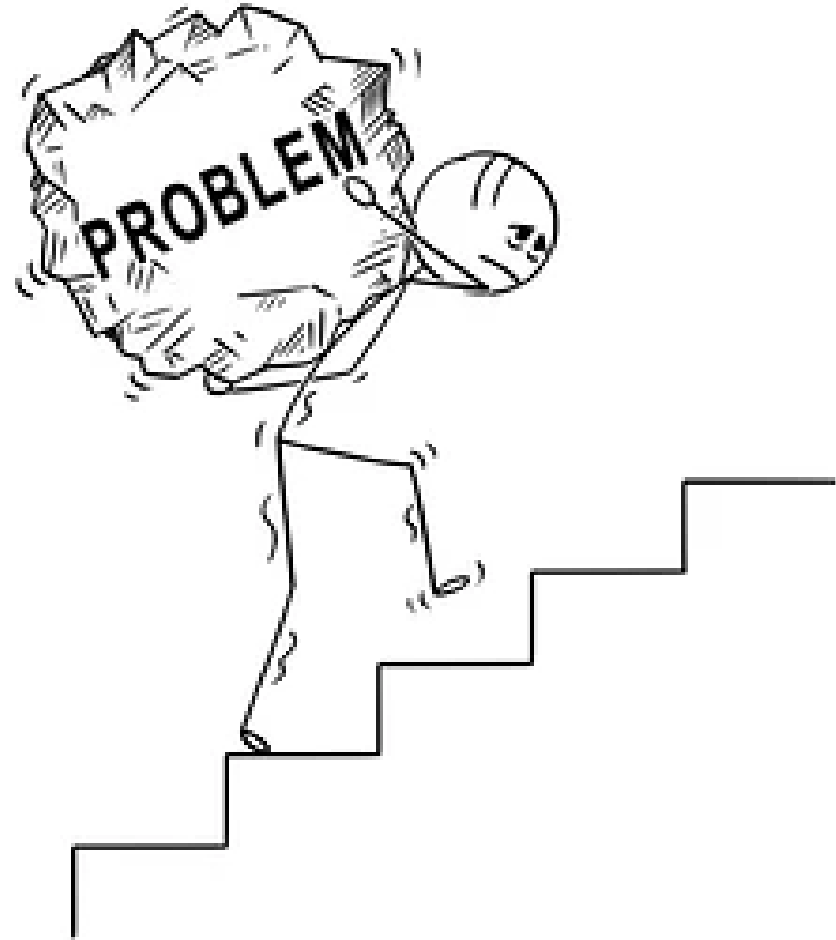
**Pepsin supplier**

# Our little problem

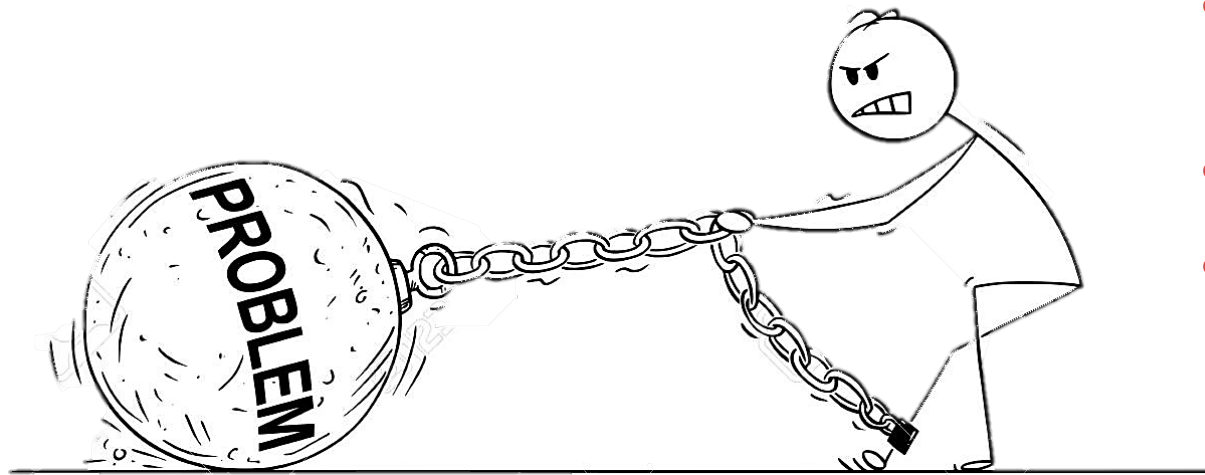


# If you are experienced

- Takes much **longer** to read the sample
- Cause **fatigue**
- We have had days with 1000 samples and 5-6 such cases



# If you are a beginner



- Takes much **longer** to read the sample
- Cause **fatigue and frustration**
- Risk of **human error or bias**

The good news is ...



**There is a**



# What ISO 18743:2015 says

- **NOTE 3** If the analyst considers that the digest fluid is not clear enough to be examined, a washing step can be performed (add 30 ml of tap water, allow the sediment to stand for 10 min, and repeat 9.7 c)

## ISO 18743:2015(E)

**NOTE** Since the sieve is used to separate muscle debris from *Trichinella* larvae after digestion, not to measure, the mesh size does not need to be periodically calibrated.

### 9.6 Sedimentation of the digest fluid in the separatory funnel

Allow the digest fluid to sediment for 30 min.

**NOTE** Although not necessary, gentle tapping of the funnel wall (e.g. every 10 min) can facilitate the larvae settling to the bottom of the funnel.

### 9.7 Collection of the primary and secondary sediment

- a) Dispense approximately 40 ml of the digest fluid (primary sediment) into a glass tube.

The stopcock of the separatory funnel shall be fully and quickly opened to ensure that no larvae are trapped on the edge of the opening or fail to be flushed out due to low flow velocity.

**NOTE 1** If the volume of the primary sediment is too small, larvae might remain in the digest fluid in the separatory funnel and might be lost. Conversely, there might be excessive debris affecting clarity if the volume of the primary sediment is too high.

- b) Allow the 40 ml of primary sediment to stand for 10 min.

**NOTE 2** If the sedimentation time is too short, larvae might not have enough time to sediment and might be lost.

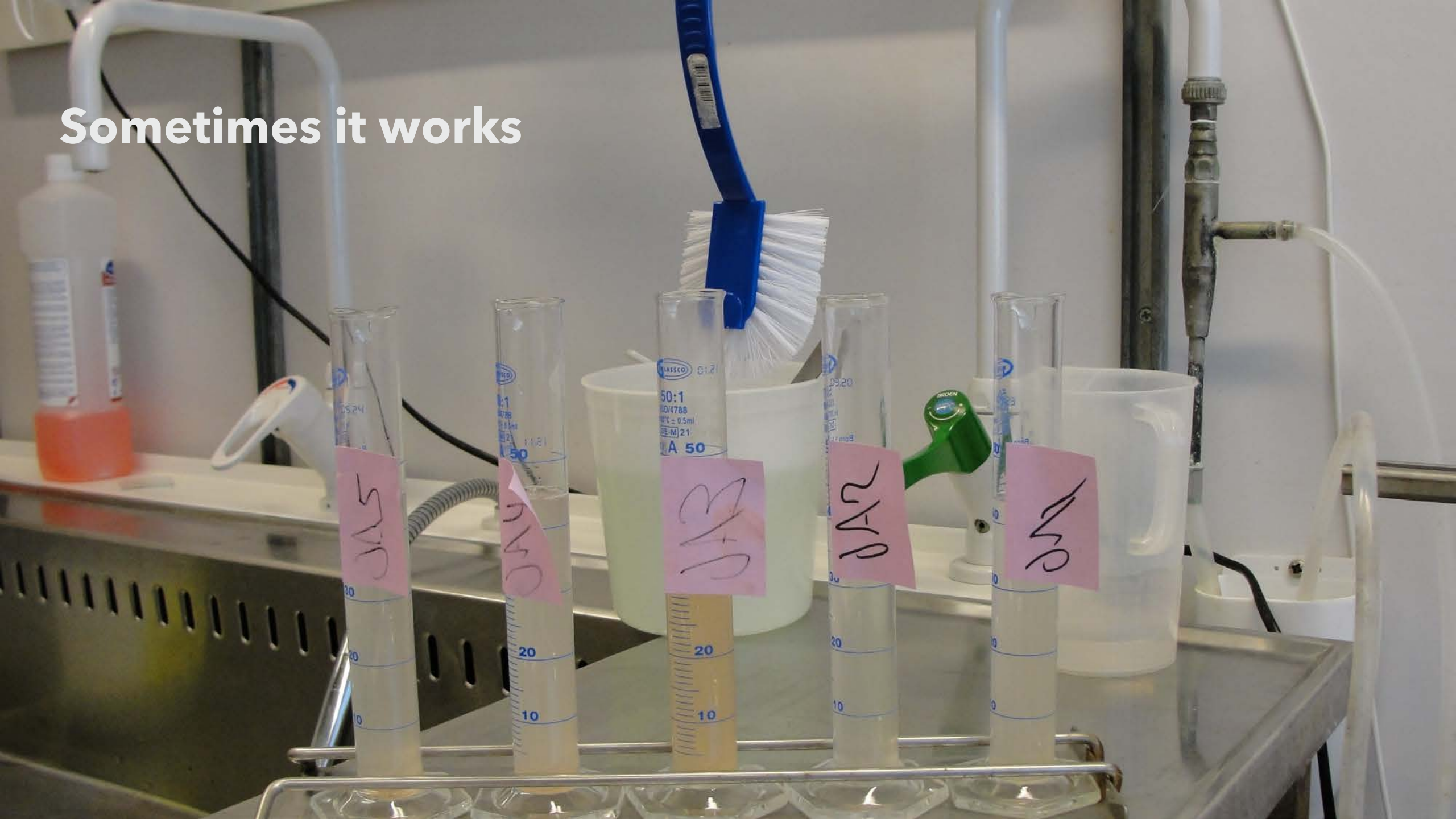
- c) Carefully withdraw 30 ml of supernatant by suction from the top of the fluid, leaving a volume of not more than 10 ml (secondary sediment).

**NOTE 3** If the analyst considers that the digest fluid is not clear enough to be examined, a washing step can be performed (add 30 ml of tap water, allow the sediment to stand for 10 min, and repeat 9.7 c).

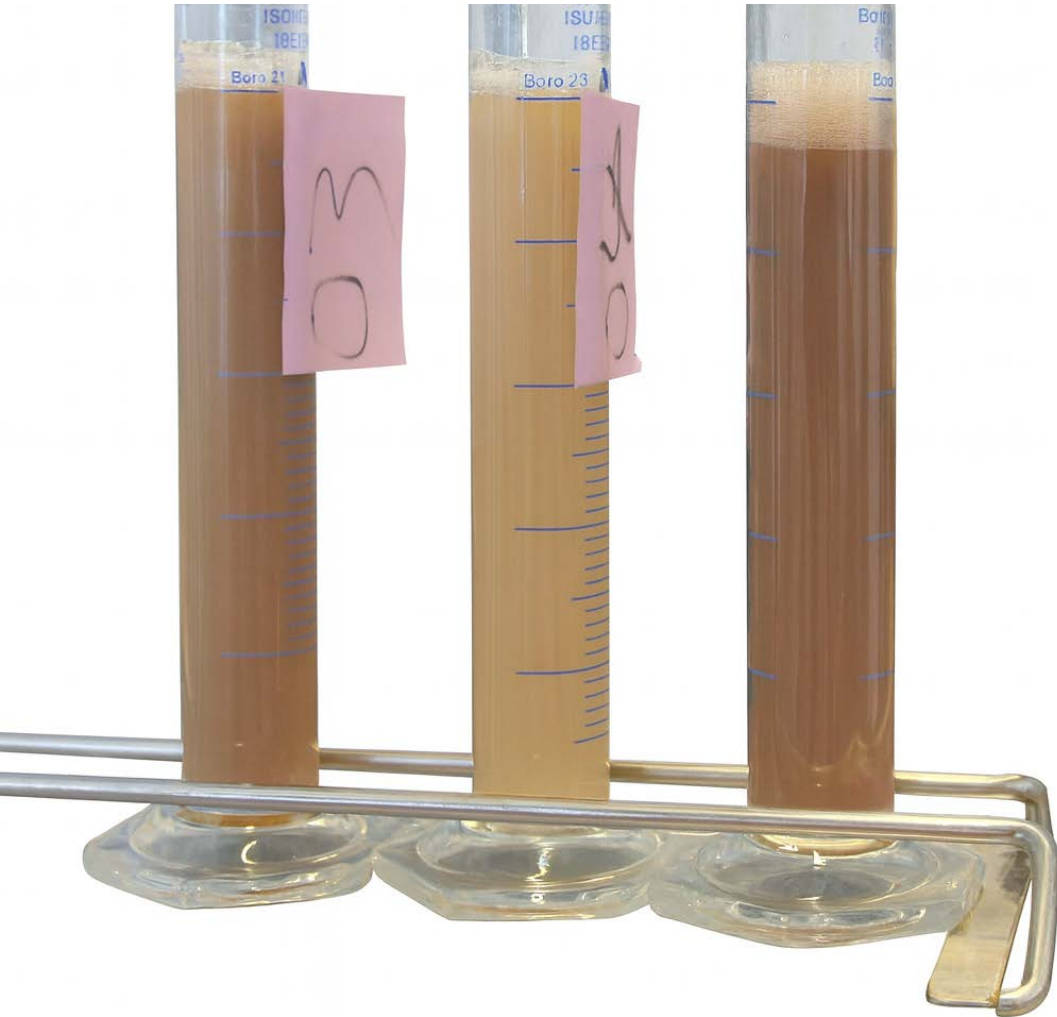
- d) Pour the secondary sediment into a gridded Petri dish or larval counting basin. To remove larvae which can adhere to the inner surface of the glass, the tube should be rinsed with 10 ml of water which is then added to the Petri dish.

- e) The final 20 ml of digest fluid in the Petri dish shall be allowed to stand for at least 1 min for any larvae to settle before microscopic examination.

Sometimes it works



# Sometimes it does NOT



- Even after 3-4 cleaning steps.

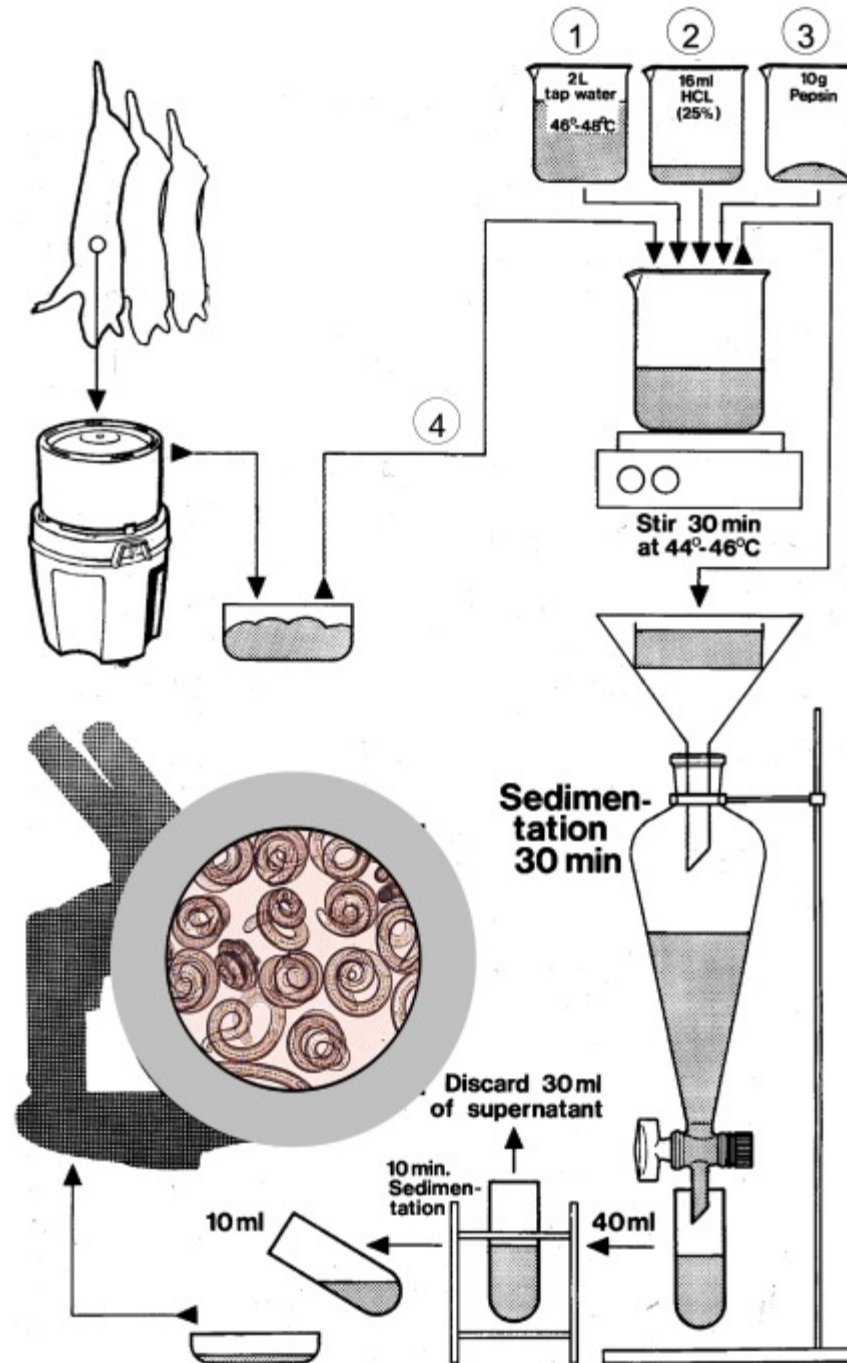
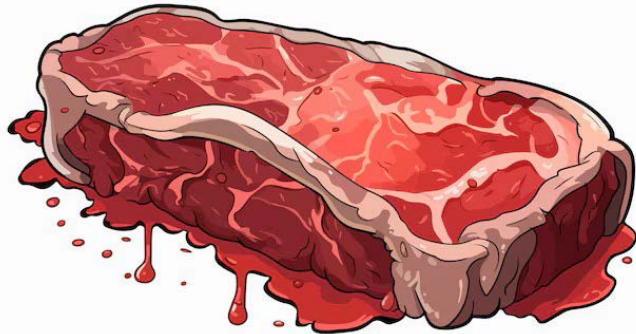
## Objective

**To evaluate a modified cleaning step designed to improve clarity and larval recovery compared with the ISO 18743:2015 method**

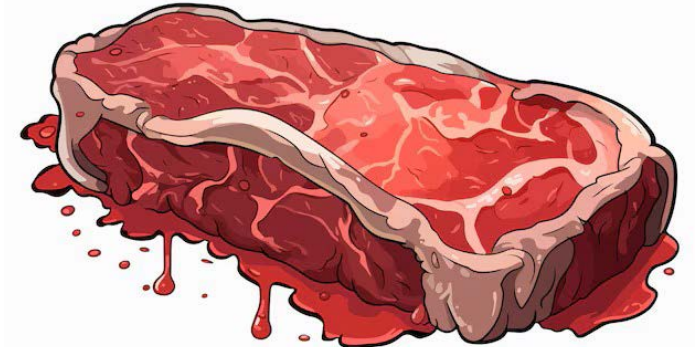


## Method

**19 run (380 samples)  
ISO method**



**19 run (380 samples)  
In-house method**





# Results

| Sample   | 01 | 02 | 03 | 04 | 05 | JA1 | JA2 | JA3 | JA4 | JA5 | SA1 | SA2 | SA3 | OS4 | OS5 | OS6 | OS7 | OS8 | OS9 |
|----------|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ISO      | 10 | 7  | 4  | 9  | 1  | 8   | 9   | 1   | 9   | 5   | 5   | 4   | 6   | 1   | 3   | 1   | 7   | 0   | 1   |
| Modified | 9  | 7  | 10 | 6  | 7  | 9   | 9   | 10  | 8   | 9   | 8   | 7   | 9   | 6   | 6   | 4   | 7   | 0   | 3   |

**Mean ± SD (ISO):** 4.8 ± 3.3      **Mean ± SD (Modified):** 7.1 ± 2.5      **p = 0.0037**

# Take-home message

- Improves clarity.
  - Speeds up reading.
  - Increase larval recovery.
- 
- Your thought?

