

Multispectral direct detection of *Trichinella* larvae on nylon filters

Kapel CMO¹, Henriksen L², Gleerup C², Bruun JM¹, Dalum L², Fischer C¹, Carstensen JM²

¹ Department of Agriculture and Ecology, Faculty of Life Sciences, Copenhagen University, Frederiksberg, Denmark.

² Videometer A/S, 2970 Hørsholm, Denmark.

Introduction

Several hundred millions EURO are spent every year to inspect meat of pigs which are primarily raised on confined industrialized farms with hundreds of animals having no real risk of acquiring trichinellosis. The same is the case for a number of regions in the world with industrialized pig production. For several decades, human trichinellosis in EU has always been caused by game meat or by meat from livestock raised outdoor, and never from meat from domestic pigs reared in industrialized farms. Thus, globally there is a need to cost-effectively document that pigs raised under such conditions are free of *Trichinella*, but a rational approach is complicated by the fact that the occurrence of both pig and human trichinellosis differ markedly between countries globally and even within regions.

Although several new methods for *Trichinella* surveillance is being developed and risk based herd certification is a potential alternative to classical meat inspection, *Trichinella* testing by digestion assays are still widespread and considered the gold standard.

A new vision-based method and instrumentation has been developed for the direct detection of *Trichinella* larvae recovered from a range of digestion processes used in classic meat inspection. The process allows for subsequent molecular analysis and thereby a thorough risk analysis of potential findings.

Instrument description

The instrument consists of a table-top scanner unit (camera, motor systems, optical and illumination components, and control system), and a PC which is running the application software.

The user prepares the sample on a dedicated sample holder (35µm net), inserts the sample into the instrument and activates the software. The instrument then scans the entire surface of the net, capturing several multispectral images, which are analysed to form a combined analysis of the entire sample.

The result of the analysis is displayed on the screen after a couple of minutes.

References

- [1] Carstensen, J.M., Folm-Hansen, J., An apparatus and a method of recording an image of an object. Patent family EP1051660, Issued in 2003.
[2] Kapel CMO, Hammeken N, Carstensen JM, Dalum L. A method and a system for detection of *Trichinella* larvae in meat samples. Patent application WO02006034716, 2006.

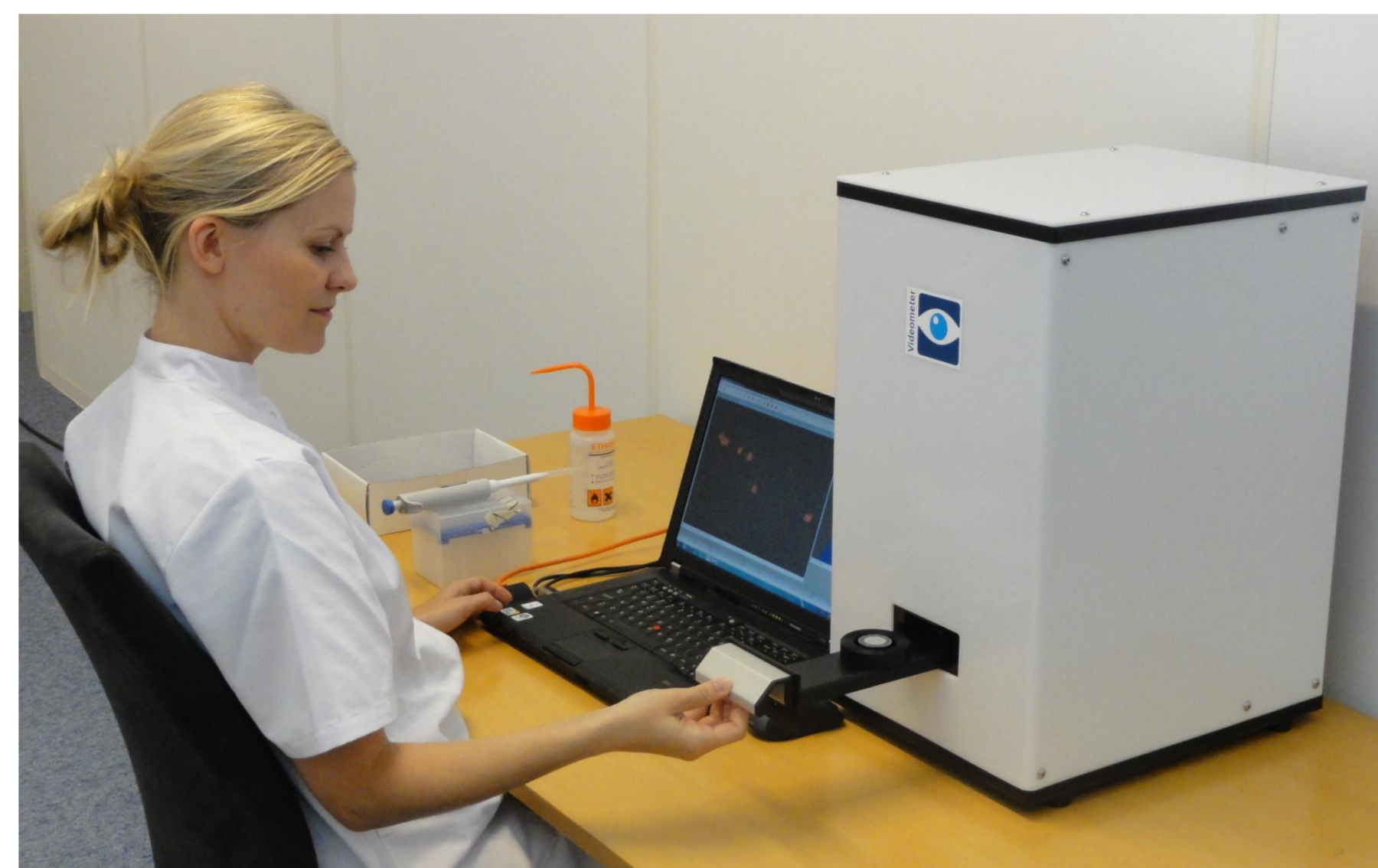
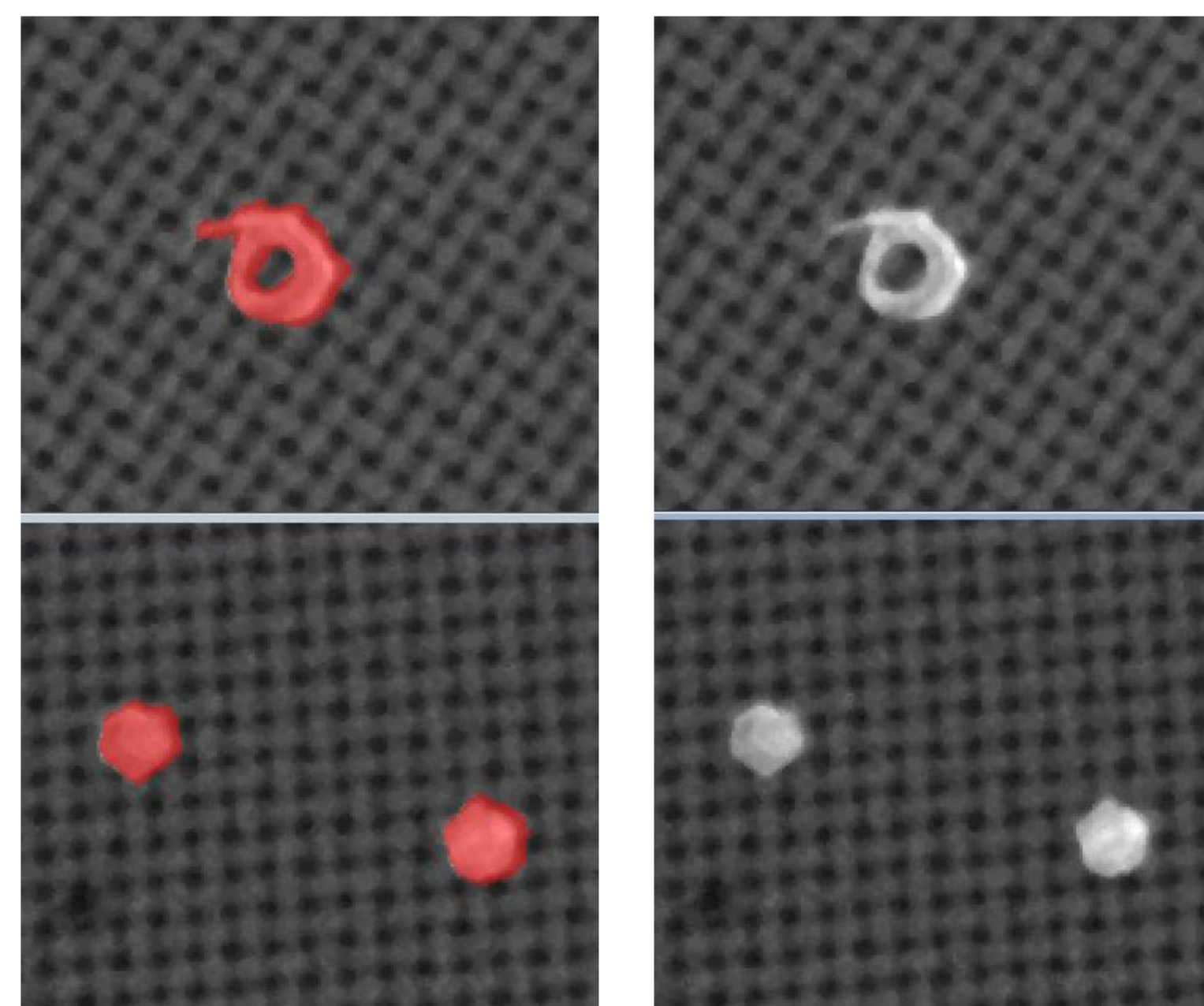


Image acquisition

The core of the instrument is a multispectral vision unit which is specially constructed to acquire the imagery^[1, 2]. It has a variety of light sources with different wavelengths and number of specially selected filters for combined reflectance and fluorescence measurements. The illumination, filtering, multispectral image acquisition of the sample, and analysis is set up in a recipe using a standard PC.

Image analysis

The images captured are processed using dedicated analysis algorithms. The analysis reports an image where each pixel is labelled a *Trichinella* or not, see figure below.



The areas detected as *Trichinella* are marked with a red overlay. From the figure it is also noted that the *Trichinella* may appear in both tightly spiralled 'knots' as well as in more loose spirals, resembling a 'donut'. C-shaped larvae was detected in the same way.

Test material

Trichinella spiralis larvae were propagated in mice and released by artificial HCl-pepsin digestion.

Samples were prepared from:

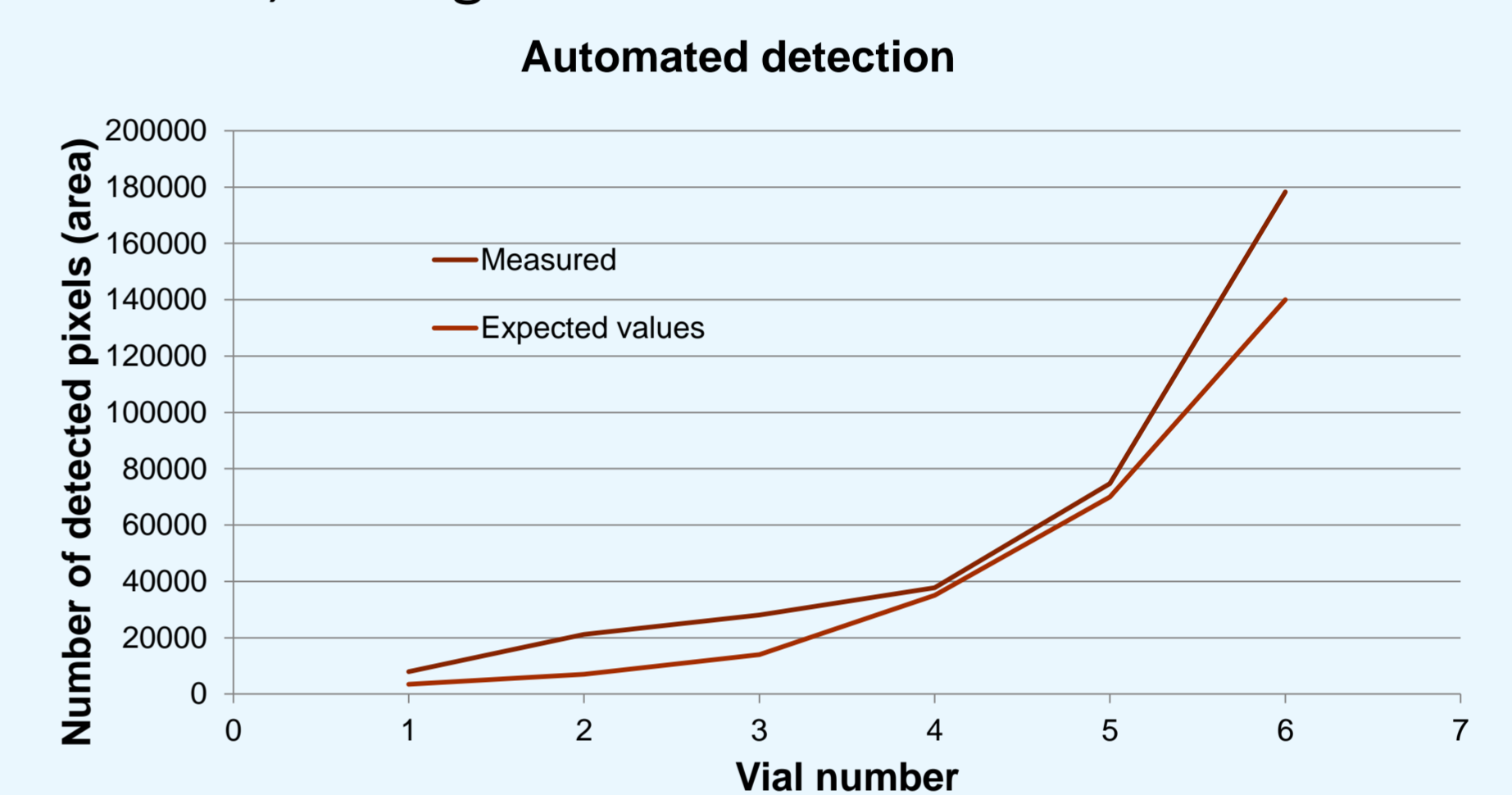
- 1) A series of vials with dilutions of 0, 10, 20, 40, 100, 200 and 400 larvae/ml in water.
- 2) A series of vials with dilutions of 0, 10, 20, 40, 100, 200 and 400 larvae in digestive fluid with partially digested muscle fibres.
- 3) Two series of vials with accurate number of larvae picked individually: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 larvae

Performed tests

The test material was presented to the instrument over 4 consecutive days by 3 different operators. The full spectral images were stored for all samples and subsequently analysed on the computer.

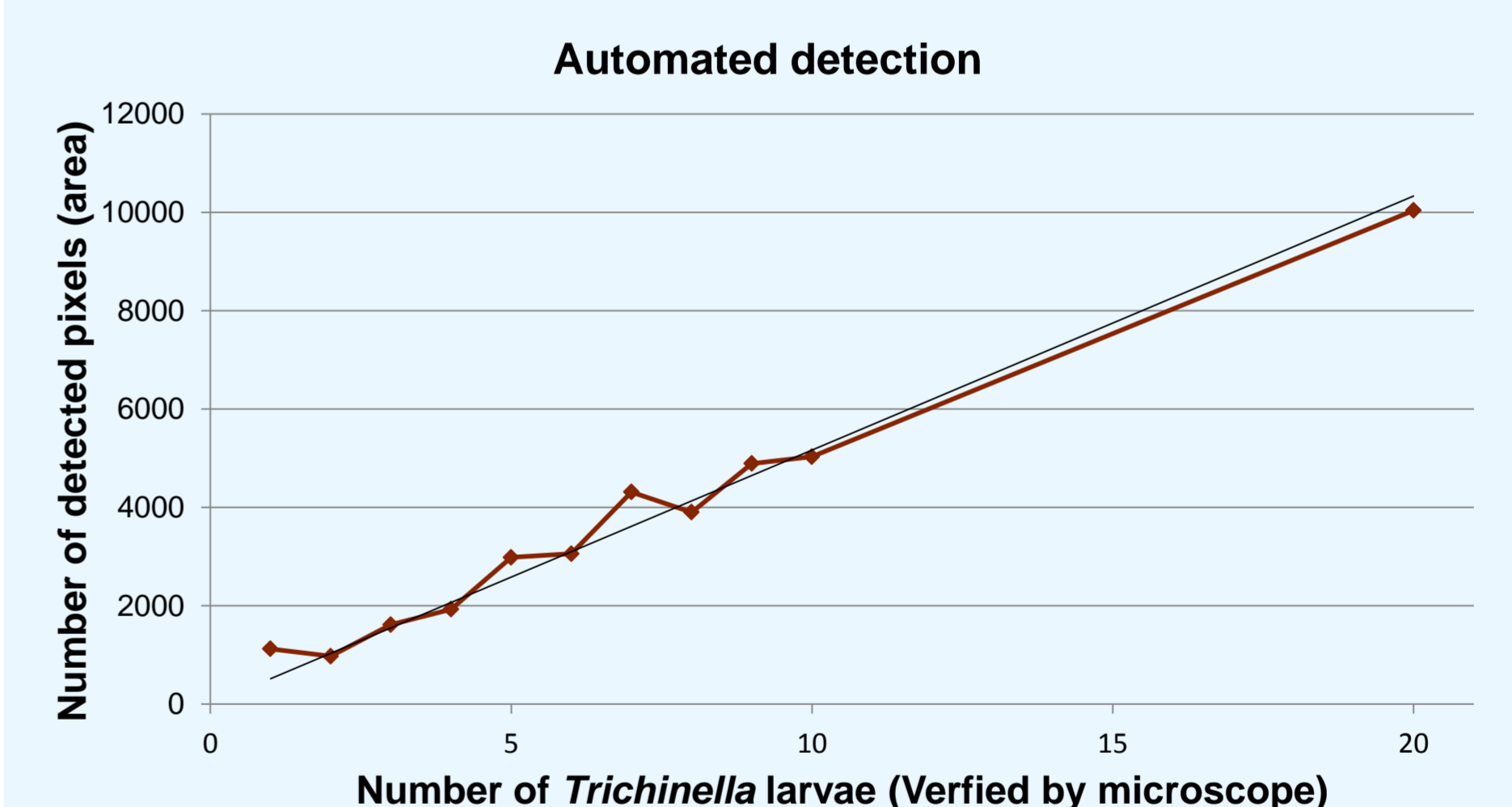
Proof of concept analysis and results

The result of sample set 1 dilutions displays good correlation between manual and automated detection, see figure below.



The detected and expected amount of *Trichinella* are not completely identical, as sub-sampling from a dilution is not 100% accurate.

In sample set 3, the number of *Trichinella* on the tested nets was verified manually by microscopy. The number of pixels detected as *Trichinella* by the machine was in close correlation to number of *Trichinella* confirmed by microscopy, figure below.



A few samples with less coiled *Trichinella* produced a higher number of pixels per larvae as they cover as larger area, which may influence the precise quantification of larvae.

At higher larvae number, a few samples produced more pixels than anticipated, but such "false positive" observations do not constitute a hazard to the end user and may be suppressed in later versions of the analysis software. More importantly, no false negative test were observed.

Conclusions

There is a close correlation between the manually verified number of *Trichinella* larvae recovered on a net and the number of pixels detected as "positive" by the instrument.

The instrument is able to detect single larvae even in presence of muscle fibres.

False positives will not compromise consumer safety. No false negatives have been encountered.