BoarCheck

A study on rapid methods for boar taint used or being developed at slaughter plants in the European Union

D5.2 Final Report

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### Abstract
This report summarises and describes the main results and conclusions of a study on rapid methods for boar taint detection of entire and immunocastrated male pigs used or being developed at slaughter plants in the European Union. In addition, recommendations for further work in this field are given.

Both instrumental and sensory based (human nose) methods have been considered in this study. At industry level, different sensory based methods (human nose method) have recently been implemented for use at the slaughter line at major slaughter companies in the European Union. Only one instrumental method, the Danish colorimetric (skatole equivalents) method is being used at one slaughter line in Denmark.

Various instrumental methods and measurement principles are still in the development stage, among which a few were identified to have the potential for boar taint detection at industrial level. Therefore two of the most relevant instrumental methods were tested.

Based on the collected method information, critical review and testing of the most relevant methods, and taking also into account the industrial method requirements, there is currently no dedicated instrumental measurement system available for on/at-line sorting of boar tainted carcasses that measures both androstenone and skatole.
Accordingly, there is still development and method validation needed, which applies to both sensory based and instrumental methods, before it can be concluded that they comply with all the industrial method requirements.

A critical issue is still how to establish objective criteria for defining a verifiable reference standard for boar taint, and which the future sorting methodology has to comply with. However, acceptance levels may vary between countries, product types and customers, that would require different threshold levels of androstenone and skatole for sorting purpose. Currently the only objective measure of boar taint is a chemical analysis of the content of androstenone and skatole.

In conclusion, since there are still no properly validated detection methods that comply with the industrial method performance requirements for the detection of boar taint, currently no short list of selected rapid detection methods could be provided.
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1. Project scope and methodology

The BoarCheck project is a study that aims to give a survey and critical review of the rapid methods (used or being developed) for detection of boar taint in entire and immunocastrated male pigs at slaughter and cutting in the European Union. In addition, to compare the feasibility, performance and cost of different methods being used or being developed in pig slaughter plants and to identify the most relevant methods. This has been achieved by the following progressive tasks:

- collecting up to date information on existing methods and methods under development, by a review of the existing literature in the field and surveys of the relevant slaughter plants and laboratories
- conducting a feasibility and cost assessment study of the various available methods
- organizing a workshop gathering industrial and scientific participants to discuss the results of the surveys and of the study on feasibility and cost assessment and select a few most relevant methods
- conducting an objective comparison of the selected methods. The comparison will include reference to sensory methods using a trained panel and the levels of boar taint compounds, using laboratory techniques that will be validated against the reference method being developed at JRC/IRMM

The generated information and conclusions of the study should provide the EU Commission with information on the most suitable and reliable detection methods applicable to slaughter plants for sorting boar tainted pig carcasses at the slaughter line.
2. BoarCheck project Consortium

The BoarCheck project consortium was comprised by the following 8 participant organisations:

**Nofima**: The Norwegian Institute of Food, Fisheries and Aquaculture Research, Norway

**ILVO**: Instituut voor Landbouw- en Visserij Onderzoek (Institute of Agricultural and Fisheries Research), Belgium

**IRTA**: Institut de Recerca i Tecnologia Agroalimentaries (Institute of Agrifood Research and Technology), Spain

**DMRI**: Danish Meat Research Institute, Denmark,

**IFIP**: Institut de la Filière Porcine (French Pork and Pig Institute), France

**DLO**: Stichting Dienst Landbouwkundig Onderzoek, The Netherlands

**UGo**: Univerity of Goettingen, Germany

**UWE**: University of the West of England, United Kingdom
3. Executive summary

3.1 State of the art rapid detection methods

Some basic generic criteria were considered to make boar taint detection methods qualified as potential candidates for further evaluation during the BoarCheck project:

- The method should have sufficient selectivity and sensitivity to be fit-for-purpose for the measurement of boar taint
- It should preferably integrate the measurement of relevant boar taint marker substances in a single system
- The method should be highly correlated to consumer perception of boar taint
- The method should give results immediately or shortly after the measurements (preferable within minutes)
- The method should have high sample throughput capacity
- The method should be cost effective.
- The method should have a capacity to be incorporated/integrated in current industrial setting

Method information has been collected and critically reviewed with regard to relevance and potential for future use as rapid detection systems for sorting boar tainted male pig carcasses at industrial level. It should be highlighted that some relevant methods, which are currently under development, had limited information about performance characteristics in the public domain because of the Intellectual Property issues. The method performance assessment of existing methodology and prioritization of the most promising methods with future potential for boar taint detection at industrial level was based on (i) information available in the public domain; (ii) discussions under confidentiality agreement terms.

Several potential rapid methods were identified that have relevance and a potential for boar taint detection at industrial level. This applies to both the analytical and sensory based methods. The human nose methodology can be regarded as a possible short term solution, but instrumental methods at industrial level may be more desirable on a long term.

In addition, a method performance parameter list based on quantitative criteria was established with emphasis on the industrial method requirements, which was used as a basis for selecting the most relevant and suitable methods with a future potential as boar taint detection system. Both instrumental and sensory (human nose) based methods were considered. Industry requirements were determined as a part of this project using a specifically designed questionnaire and discussion groups during an industry-orientated workshop.

For several of the identified methods considered to have a potential for further development for use at industrial level, performance characteristics and validation data are not sufficiently documented. The same applies also to the methods which are still in development and have not been validated or tested in industrial setting. Also several of the method studies represent only limited feasibility studies on the measurement principle suggesting the measurement to have potential for this application, but have not yet been
fully validated at this stage. And they are still suffering from the lack of proper and standardized sampling that still needs to be integrated with the measurement unit.

### 3.1.2 Instrumental methods

All identified relevant instrumental methods are still at the research and development stage. Many of these methods and measurement principles represent only limited feasibility studies suggesting that they may have a potential for this application. Some methods where sufficient performance data are available could be ruled out for further consideration since they did not comply with the established performance criteria. In particular, these methods do not fulfill the industrial requirements for analysis time or simplicity with regard to both operation and complexity in technology (for example high resolution mass spectrometry). Instrumental methods based on mass spectrometry which have the ability to specifically measure both skatole and androstenone simultaneously seem to fulfil most of the industrial method requirements. In addition, the electrochemical sensors-based method and insect-based biosensing were identified as methods which also have a potential to fulfil the industrial requirements. However, these methods are also lacking sufficient documentation on performance and validity that would require more testing before they can be considered for further implementation at industrial level.

For most methods under development, the sampling is still an issue, and which is a critical element for the industrial application, and that would require that further work is conducted to adapt and validate a proper sampling system to the respective measurement technology.

### 3.1.3 Human nose method

The performance of the sensory methods with regard to industrial method specifications still needs to be properly validated and the practical feasibility and costs need to be assessed. Provided that the accuracy is good enough (which needs to be further documented), this methodology may represent a solution for the detection of tainted carcasses at the slaughter-line. It was therefore recommended that this methodology is further tested and considered for the method comparison study taking place during WP3 of this project. Also different method protocols including training of assessors, sampling, and criteria for assessing tainted samples are being used. It should be evaluated to what extent it may become standardized for future routine sorting of tainted male pig carcasses. A first prerequisite for good boar taint evaluation is the sensitivity of the experts towards androstenone. The next step is a thorough training of the experts to detect boar taint or off odour due to skatole and androstenone.

Different sensory methods have been developed and are being used at several slaughter plants. Further, it has been documented during the review that there is still research and development and method validation needed, which applies to both sensory based and instrumental methods, before it can be concluded that they comply with all the industrial method requirements.

Sensory methods (human nose) complied with requirements for all performance parameters with the very important exception of accuracy (sensitivity and specificity) which is mostly not documented or poorly documented. The scarce evidence does not allow concluding on the performance of sensory evaluation.
3.2 Industrial situation

As one of the project objectives, an analysis of industry needs and requirement in a boar taint analysis method was conducted using a questionnaire (Annex 1).

Most of the pig slaughtering companies in the EU that responded to the questionnaire, indicated to slaughter entire male pigs or immunocastrates. Boar taint detection systems are in use or under development in several countries, such as Belgium, Denmark, France, Germany and the Netherlands. These countries only recently started to slaughter entire male pigs in large numbers. Countries, like Spain and United Kingdom (UK), that have been slaughtering entire male pigs in large numbers for longer do not have online boar taint detection systems. Boar taint detection was an issue for the EU pig slaughter companies rather than for the pig slaughter companies in third countries.

Currently, sensory based/human nose methodology is being used at several slaughter lines at the major European slaughter companies (Denmark, Germany, The Netherlands, France and Belgium) as a routine sorting method, and at some slaughter companies this methodology is still in development. Only one instrumental method, the Danish colorimetric (skatole equivalent) method is being used at one slaughter line in Denmark. Nine out of ten slaughter companies had a rapid detection system for boar taint based on sensory analysis, and one based on chemical analysis of skatole. Five of the 32 EU based slaughter companies, located in Spain and Belgium, indicated that no boar taint detection system was needed. For all sensory methods, all entire males were assessed at the normal slaughter line speed by heating subcutaneous fat. The three companies that indicated to receive boar taint related complaints only received a few per month. Some companies had customers who refuse meat from entire male pigs, such as specific retailers, butchers or the Japanese market.

Other aspects, such as location of the detection system, heating method, and evaluation of method performance varied between slaughter companies. The indicated requirements to a future boar taint detection system of slaughter companies are similar to the characteristics of the existing systems.

The requirements for the pig slaughter industry for on/at-line application of measurement technology at the slaughter line were further defined in Work Package 1, Task 1.3 (Definition/identification of industrial method specifications) based on information provided by the slaughter companies (WP1, Task 1.2, Survey of methods used and in development) with emphasis on carcass sorting criteria (rate of false negatives and false positives), method performance criteria (sample throughput, analysis time, reproducibility, accuracy, sensitivity etc.), automation, plant characteristics (e.g. plant size). The overall requirements are that an increased production of entire male pigs does not discourage consumers from buying pork, and that a detection system does not increase production costs substantially.

In order to fulfil these overall requirements for the slaughter industry, minimal standards on a number of system attributes were defined: 1) Requirement for set-up and operational costs. These will depend on the size of the slaughterhouse. 2) The system should include registration of individual carcasses, possible sampling, measurement, logistics of sorted carcasses and be integrated with the IT system. 3) The system must be capable of being scaled to a situation where male pigs no longer are castrated in the EU. 4) The measuring results must be valid, verifiable and thereby trustworthy.
3.3 Costs

Further the costs were assessed (Work Package 1, Task 2.2, *Cost assessment of implementation and further development of existing and potential rapid methods*) for the two method strategies, i.e., instrumental and sensory. For larger slaughterhouses an instrumental method is preferable to use instead of a sensory based solution. This is due to the fact that the cost of wages for running a sensory based system will grow almost proportionally with the number of male pigs slaughtered per day. With an automated industrial system of measurement, the labour requirements are almost independent of the number of boar taint assays that need to be performed daily.

The initial startup/installation costs are very different for a sensory based system and an automated system. When slaughtering 1 000 male pigs per day the initial costs for a sensory based system and the fully automated instrumental method will be of the order 80.000 € and 680.000 € respectively rising to 220.000 € and 830.000 €, respectively at 5 000 assays per day. However, these differences are rapidly compensated for by the much lower operational costs of the automated system compared to the sensory based system.

The point of breakeven between a sensory system and an automated system is shown to be below 2 000 assays per day. Above this figure the automated system will be economically advantageous with a time to breakeven of approximately 2.5 years. However, there are currently no automated solutions available that have been tested adequately on a larger industrial scale with respect to reproducibility, long term stability, robustness to slaughterhouse environment, for measuring androstenone. These figures are derived from the comparison of two extreme methods, one sensory method requiring very high levels of labour and one instrumental method requiring very high level of investment. Points of breakeven (in terms of number of pigs per day and years for pay-back) may vary quite extensively depending on the level of labour required by sensory methods and the level of investment required in instrumental methods.

The assessments of the costs of operations given in this work do not take into account the number of misclassified carcasses either false negatives or false positives that inevitably will result from any sorting system. Carcasses measured as containing unacceptable levels of boar taint that in fact are acceptable (false positives) will be a great economic burden for the pork industry. The opposite where carcasses containing unacceptable levels of boar taint are judged as being acceptable (false negatives) will result in loss of consumer confidence in the pork industry.

A summary of the evaluation of costs, as calculated in part 2, is provided. Both operational costs and installation costs are calculated per tested carcass. The total costs (operational + installation) were evaluated around 0.2, 1.0, 0.3 and 0.4 € per tested carcass for on-line sensory, at-line sensory, on-line instrumental and at-line instrumental methods, respectively. These costs do not include chemical, reagents and maintenance. They comply with the cost requirements stated in part 1 (< 2 € per tested carcass).

3.4 Workshop

An international workshop on rapid detection methods for boar taint for the pig industry and research communities (WP3) was organised in Monells (Spain) on 4 December 2013. The
workshop focused on critical discussions of the results and conclusions of WP1 and WP2. Three discussion groups were set up, two on sensory methods, one on instrumental methods. Sensory methods, including the human nose methods currently used in some slaughterhouses, were considered as useful but there were concerns about their accuracy which is poorly documented in the scientific literature. It was widely held that there is no need for harmonisation of the methods, but that the way to assess their performance and accuracy should be harmonised. There was a lot of debate on the gold standard that should be used to assess accuracy. A majority of participants were in favour of using androstenone, skatole and indole levels but this view was challenged by others on the ground that these compounds do not explain the whole of boar taint. Single experts were ruled out as gold standard. Expert panels could be envisaged but harmonisation of the panels would be very difficult. Since ideally, the gold standard should be established based on consumer acceptance thresholds, this has also shown to be complicated, as demonstrated during the DG Sanco CAMPIG project (Study on consumer acceptance in the European Union and in third countries of pig meat obtained from male pigs not surgically castrated), which at tried to define the consumer acceptance/threshold levels for the boar taint substances, skatole and androstenone. Despite a sound and comprehensive methodology, the CAMPIG study concluded that single threshold values for androstenone and skatole could not be specified.

The recommendations from the workshop were i) the accuracy of human nose methods should be assessed, using both ASI levels and expert panel as reference; ii) a discussion between academic and industry scientists should take place to set up a scientifically sound revised protocol for measuring the accuracy of human nose methods; iii) perform a measurement of the accuracy of any instrumental method willing to be evaluated (no short list established). Recommendations for longer term studies were also given.

### 3.5 Selection of prioritized methods

For the selection of relevant methods, the degree of compliance with the established industrial requirements for the various performance parameters was used. The relevance of the methodology was then assessed according to three compliance categories: OK, i.e., complies with requirements, does not comply with requirements or not documented or poorly documented.

A few methods were identified with a future potential for sorting out boar tainted carcasses at the slaughter line and which are recommended for further development and testing to meet industrial method specifications. Based on a critical review of the collected method information and its compliance to the industrial method performance requirements, the following methods were found to be most relevant:

- Human nose
- Mass Spectrometry (MS)-based techniques:
  - SIFT-MS (Selected Ion Flow Tube Mass Spectrometry)
  - RF-MS (Rapid Fire Mass Spectrometry)
  - PTR-MS (Proton Transfer reaction Mass Spectrometry)
- Insect based biosensing
- Electrochemical sensors
It has, however, been difficult at this stage to make an appropriate assessment of their suitability and reliability to establish a ranking list of the most promising methods since they have not yet been fully validated. However, these methods seem to have relevance and may fulfill the method requirements necessary to be regarded as potential boar taint detection at industrial level. However, more research, development and validation work as well as the development of effective sample collection approach would be needed to get a sufficient documentation to prove their fitness for use at industrial level.

The suggested list of prioritized methods was discussed during the workshop and approved for further method comparison testing.

### 3.6 Method comparison study

Research laboratories and industry were invited to participate in the method comparison study of the prioritized methods. The aim was to carry out an evaluation of the method performance of the potential methods under development (mostly instrumental methods) and methods that are currently used at industry level (human nose method). Since this required two different testing schemes, separate protocols and instructions were prepared for each method.

However, due to limited labour, time and financial resources of the invited participants for the study, only two of the selected instrumental methods could be included in the study; both were gas-phase based analysis methods, the SIFT-MS technology and the insect based biosensor methodology.

Measurements on lard with the SIFT-MS technique, showed a high mass spectral complexity even at the relatively low sampling temperature (70 °C). It was concluded that the SIFT-MS with low resolution mass spectrometric detection at its present state is not a satisfactory solution for the pork industry when rapid sampling is required.

The biosensor based method relies on an initial calibration step that requires that the insects (parasitic wasps) are trained to known concentrations of skatole and androstenone in boar fat as single compounds. Since such calibration samples were not available at the time of testing, the performance of this method could not be properly evaluated.

For the human nose method testing, a study protocol was prepared that aimed at evaluating at least 300 carcasses online by at least one trained expert assessor. Ideally, all these carcasses should be sampled and evaluated by both chemical analysis and an expert panel. However, as no budget for chemical analysis was available, possible participants were asked to pay a participation fee that would cover the cost of chemical analysis of a subgroup of 50 samples. As not all samples could be evaluated analytically, the protocol foresaw to calculate sensitivity and specificity based on the sensory evaluation of an expert panel as golden standard. The results of the chemical analysis and an extra expert panel would then provide more information regarding the match or mismatch between the online detection method and the expert panel (false positives and false negatives).

However, due to time constraints, budget constraints and concerns regarding the protocol, only one out of five slaughterhouses accepted at the end to participate in the method study.
As anonymity could not be guaranteed in this way, it was decided to withdraw the protocol and propose a second protocol evaluating at line performance. This second protocol would not enable the evaluation of online method performance, but would already provide valuable information regarding the sensitivity towards androstenone and skatole of the experts performing the human nose methodology. Besides, evaluation of at line performance enables comparison of a large group of experts and comparison of experts between countries, as the same samples can be tested by several experts and sent to several countries. Unfortunately, not all slaughterhouses were willing to cooperate in this proposal either.

This task could therefore not provide any results regarding the performance of the methods currently in use, but provides valuable protocols which can be used for evaluation of online method performance in future.

In conclusion, at the current state no short list of selected rapid detection methods to detect boar taint carcasses could be provided.

Accordingly, there is still a need for the development and validation of a reliable on-line boar taint detection method in slaughterhouses to remove tainted meat.

### 3.7 Conclusions

#### 3.7.1 State of the art rapid detection methods

Boar taint detection in slaughter plants is an important topic in the pig slaughtering sector in the EU and less in the pig slaughtering sector in third countries. In spite of the EU industry recognizing boar taint as an issue, slaughter companies in the EU indicated receiving only a few boar taint related consumer complaints monthly. This may also indicate that some slaughter companies succeed in marketing entire male pigs under a quality assurance scheme that includes detection for boar taint in a manner that satisfies their clients in retail and out of home markets.

There is currently no instrumental method available for use at industry level that measures the two main compounds responsible for boar taint, namely skatole and androstenone. Among the instrumental methods that exist at laboratory level, only a few have a good potential in terms of capacity and reporting time.

At industrial level, large pig slaughter companies in the EU are using or developing a boar taint detection system. Several major slaughterhouses in the EU (Germany, The Netherlands, Belgium and France) have applied the human nose methodology at- or on-line for sorting boar tainted carcasses. Due to the economic scale, smaller pig slaughter plants could have difficulties in implementing such a system.

Providing that the accuracy of the human nose is good enough (which needs to be further documented), sensory quality control may represent a provisional solution for the detection of tainted carcasses at the slaughter-line. Currently, the human nose methodology should be regarded as the only available possible short term solution, but instrumental methods at industrial level are desirable in the long term as they are anticipated to be more cost-effective when compared to human nose.
Commercial slaughterhouses at present do not have other available alternatives than the human nose method, but the economic potential of instrumental methods is beyond doubt. This calls for establishing an infrastructure in which business and science work together to define performance indicators for current and future detection methods.

**In conclusion, at the current state, no short list of selected rapid detection methods to detect boar taint carcasses could be provided.**

### 3.7.2 Instrumental methods

On the basis of the identified industrial method requirements, quantitative standard method performance criteria were established for the selection of the most relevant instrumental methods currently in development. A few of these methods may have the potential to be able to comply with industrial method requirements.

However, due to limited time, labour and restricted financial resources among the invited participants, only two of the most relevant instrumental methods identified could be included in the method comparison study: The SIFT-MS and the insect based biosensor method. Both methods rely on detection of the boar taint substances in the gas phase.

Measurements on lard with the SIFT-MS technique, showed a high mass spectral complexity even at the relatively low sampling temperature (70 °C). When the already high background levels at the target compound product ion masses are considered, together with heating samples to >160 °C to ensure significant androstenone is volatilised and sample carryover minimised, it was concluded that SIFT-MS with quadrupole-based detection is not a satisfactory solution for the pork industry when rapid sampling is required.

The insect-based biosensing method relies on an initial calibration step that requires that the insects (parasitic wasps) are trained to known concentrations of skatole and androstenone in boar fat as single compounds. Since such calibration samples were not available at the time of testing, the performance of this method could not be properly evaluated; neither nor its long term performance could be demonstrated.

### 3.7.3 Human nose method

The sensory based human nose methodologies have been developed and are being applied at the slaughter line and comply with the requirements to analysis capacity and time. Provided that their accuracy is good enough (which needs to be further documented), this methodology may represent a solution for the detection of tainted carcasses at the slaughter-line at least in the short-term.

However, the accuracy (sensitivity and specificity) of the human nose methods as they are currently in use in several slaughterhouses in Europe, is poorly documented. Therefore these methods need further validation to prove their fitness.

Different method protocols including selection and training of assessors, sampling, heating and criteria for assessing tainted samples are being used. The experiences so far from the European industrial human nose trials have addressed the need for further method development regarding both selection and training of assessors to improve the performance.
of the method. Besides, it would be useful to evaluate and compare method performance on-line as well as off-line.

The human nose methodology was also intended to be performance evaluated at industry level during WP4. However, due to tight deadlines and concerns regarding the protocol, only one out of the five slaughterhouses was willing to participate in the study.

There is currently no general agreement on the use and effectiveness of the sensory methods applied by the industry.

3.7.4 Definition of the boar taint - “gold standard”

A critical issue is that there is no generally accepted verifiable reference standard for boar taint. A lot of work is still needed to establish a universally recognized “gold standard” for boar taint. The following points need to be considered for the establishment of a sound definition of boar taint gold standard:

- The ideal “gold standard” would be the consumer perception of boar taint. In practice, it is however not possible to use it because of the large variability of consumer reactions and the extremely high cost of performing consumer studies on large number of samples. Ideally, the gold standard should be established based on consumer acceptance thresholds. As demonstrated during the CAMPIG project (Deliverable report D2.4, Recommendation-report to the EU Commission with respect to the acceptability of boar taint from non-surgically castrated pigs in the selected EU countries), which aimed at trying to define the consumer acceptance/threshold levels for the boar taint substances, concluded that single threshold values for androstenone and skatole could not be specified. Some intermediate golden standard has therefore to be established, which is well related to consumer perception of boar taint. This view is supported by the recent conclusion of the CAMPIG project;
- Acceptance levels may vary between countries, product types and customers, which would require different threshold levels of androstenone and skatole for sorting purpose.
- The most commonly used intermediate golden standard is the levels of androstenone and skatole in fat. There is substantial information in the literature regarding their relationships to consumer perception of boar taint, which is still somewhat controversial and there is no universally recognised agreement of the thresholds that should be used. Current work within the DG SANCO supported project CAMPIG should provide new insights in that respect. However, it should be noted that consumers’ perception of boar taint and therefore, related skatole and androstenone threshold levels may vary between countries and product markets.
- Other criteria, such as sensory assessment by expert panels (or by an extremely good expert, which is however not the same as a very sensitive expert), could also be used as golden standard for boar taint, provided that they are sufficiently related to consumer perception of boar taint.
- The level of customer complaints is often used by the industry to assess the validity of a boar taint detection method. Although this is perfectly understandable at a very global level, this cannot be used for a scientifically acceptable evaluation of sensitivity and specificity.
3.8 Recommendations

3.8.1 Instrumental methods

- The study has shown that there are a number of instrumental methods under development which can potentially be applied for on-line detection of boar taint. However, further research on the development and full validation of these methods under industrial conditions is required before they can be considered for application in an industrial setting.

- To perform an evaluation of the accuracy and sensitivity of the instrumental methods under development, as described in BoarCheck Work Package 4. However, sufficient financial and personnel resources should be provided in order to enable such evaluation against currently used chemical analysis of skatole and androstenone as well as sensory analysis.

- Instrumental analysis should be compared to sensory analysis such as conclusions on the agreement between both types of quality control can be drawn.

- Since boar taint acceptance levels may vary between countries, product types and customers, that would require different definition of threshold levels of androstenone and skatole for sorting purpose, instrumental methods should be able to handle various threshold levels.

3.8.2 Human nose method

- The accuracy of human nose methods should be evaluated at industry level. The proposal in BoarCheck Work Package 4 describes a protocol to achieve that, using AIS levels as gold standard for boar taint, and in addition sensory assessment with a trained expert panel. However, sufficient financial and personnel resources should be provided in order to enable such evaluation against currently used chemical analysis of skatole and androstenone as well as sensory analysis.

- The performance of the human nose methodology is poorly documented. We recommend that the results of the human nose methods are validated for performance as suggested in the method comparison study report (D4.1, Method comparison study) in order to determine their sensitivity and specificity under real industrial conditions. This would also require sufficient resources allocation.

- The performance of the on-line and at-line methods should be compared to evaluate the effect of the on-line conditions on the sensory evaluation of boar taint.

- Method performance of the experts performing the human nose methodology should also be compared to the method performance results of trained expert panels on-line as well as at-line, both in relation to the results of the chemical analysis in order to set achievable criteria for the human nose methodology.
The method comparison protocols prepared for evaluating the human nose methodology and evaluating the selection and training procedure of the experts performing the human nose methodology (Work Package 4) may be useful for further research in this field. However, sufficient financial and personnel resources should be provided in order to enable evaluation of method performance compared to chemical analysis as well as sensory analysis.

3.8.3 Boar taint reference standard

A verifiable reference standard for boar taint is extremely critical, both for the development of detection methods and the development of strategies to reduce boar taint.

To account for the different views on a reference gold standard, a discussion with academic and industry scientists should take place, to envisage possible ways of going around the problem of the missing universally recognised gold standard for boar taint.

There are a number of reasons why this issue should be addressed collectively, in an international project funded by the involved stakeholders:

- The research to address this issue is generic, not competitive.
- The research to be conducted involves large scale consumer acceptance studies, which are extremely costly.
- The research must be conducted at an international level as it is important to take into account variability and diversity, in eating culture, perception of boar taint, and human inter-individual variability of olfactory perception. The same methodological approach should be used in international studies to achieve reliable results.
4. Main results

4.1 State of the art of rapid methods used and in development (WP1)

4.1.1 Survey of methods used and in development

Initially (WP1, Task 1.1, Survey of methods used and in development), a survey was carried out to identify the state-of-the-art of currently used methods and methods in development for rapid boar taint detection intended for future use at industrial level in slaughterhouses. Three strategies have been applied for collecting relevant methodological information:

1. Web literature search
   A selection of relevant method key words have been defined which have been applied for the literature search on the web. Further the collected information and data have been categorised according to various measurement principles.

2. Consultancy with research groups working in the field
   A questionnaire on rapid detection methods for boar taint detection was prepared covering various methodological aspects as sampling, method performance and validation level etc. The Joint Research Centre Institute for Reference Materials and Measurement, Geel, Belgium (JRC/IRMM) also contributed to improvements of the questionnaire. The questionnaire together with a letter of intent describing the scope of the project and aim of the questionnaire was distributed by e-mail to various international research groups working in the field, manufacturers of analytical instruments and the members of the EAAP working group “Production and Utilization of Meat from Entire Male Pigs”.

3. A patent search
   Patent search on methodology has been carried out. Patent information and data have been reviewed with regard to methodology and performance. The patent search has been carried out following the three steps: Identification of keywords and concepts relevant to the scope of the patent search, search strategy, queries execution and selection of relevant patents.

Of the distributed questionnaire (Annex 1), in total, 17 completed questionnaires were received with the following distribution on measurement principles:

The measurement principles of the methods covered by the answered questionnaires covered the following measurement principles:

- Gas-phase fingerprinting/Gas-Sensor Array (3 replies)
- Mass Spectrometry (2 replies)
- Ion Molecule Reaction (1 reply)
- Immunology (2 replies)
- Electrochemical sensors (2 replies)
- Insect-based biosensing (1 reply)
By applying various combinations of the key search terms, more than 1500 hits were obtained during the web search. Most of the hits showed to be redundant and not relevant to our topic. They represent various instrumental techniques for the detection of boar taint covering different measurement principles and representing different levels of validation. Only a limited number of published scientific papers contained sufficiently substantial methodological information in order to assess their potential for future use as routine sorting tool at industrial level and have been considered in this survey.

From the 18 identified patents, eight were identified as relevant whereas patents related to genetic markers were considered as not relevant since they do not represent any detection methods for boar taint, or androstenone and skatole. In addition, three patents were identified from the received questionnaires, which recently have been submitted for filing. Since they are still in the application stage and due to IPR issues, limited information was available from these three patents.

It should be highlighted that some relevant methods, which are currently under development, have limited information about performance characteristics in the public domain because of the Intellectual Property issues. Therefore, during WP2 an assessment of existing methodology was made to identify and prioritize the most promising methods with future potential for boar taint detection at industrial level based on both information available in the public domain and discussions under confidentiality agreements.

4.1.2 Current situation of rapid detection methods in slaughter plants

Of the distributed questionnaire (Annex 2), in total, 35 questionnaires were returned, of which 31 from seven EU countries (B, DK, F, NL, G, ES, UK) and four from three third countries (Canada, New Zealand, Norway). No responses were received from slaughter companies in Italy and USA. The responding slaughter companies varied in size, with weekly total pig slaughter numbers from 800 to 400,000, and 40 to 60,000 entire males.

Current situation: market and boar taint detection

Twenty four of the slaughter companies stated that they slaughtered entire male pigs. These were located in Belgium, Denmark, France, Germany, the Netherlands, Spain, the United Kingdom and New Zealand. Three companies indicated to expect to start slaughtering entire male pigs next years (France and Norway). Of those who slaughtered entire male pigs, the median number of entire male pigs slaughtered per week was 1,300 with a range from 40 to 60,000. The low number reflects the four slaughter companies which were starting with slaughtering entire male pigs. In Brazil, by law, only castrated male finishing pigs were allowed for slaughter, so all male finishing pigs were castrated, and stopping castration was not an item of interest. In Canada, the discussion of stopping with castration was starting, and slaughter companies were considering starting to slaughter immunocastrates. In New Zealand, entire male pigs were slaughtered, but at such a young age, the average carcass
weight at slaughter was around 68 kg (MPI 2013) that boar taint was not yet a problem. Consequently, boar taint detection was not an issue. The Norwegian situation was comparable to the situation in the EU, with slaughter companies slaughtering or considering to starting slaughtering entire male pigs and immunocastrates, although they did not consider a boar taint detection system necessary.

To reduce the risk at complaints most companies sold meat from boars assessed as with boar taint on specific markets or used it in products that are consumed without heating or mixed with non-tainted meat. The three companies that indicated to receive complaints, only indicated to receive a few per month. Some companies had customers who refuse meat from entire male pigs, such as specific retailers, butchers or the Japanese market. In addition, one slaughter company that did not slaughter entire male pigs indicated that “all” customers refuse meat from entire male pigs.

The mean line speed of the slaughter companies that slaughtered entire male pigs was 439 pigs per hour. Out of the 24 companies that slaughtered entire male pigs, 12 indicated to have a boar taint detection system. These were located in Belgium, Denmark, France, Germany and the Netherlands. Four of these companies indicated to be in the start-up phase of the system. Paragraphs 2.3 and 2.4 provide a detailed analysis of the existing boar taint detection systems. Slaughter companies in Spain and the UK that slaughtered entire male pigs did not have a boar taint detection system. In these countries the slaughter of entire male pigs has been common for much longer than in the other countries, and apparently they never considered implementing a boar taint detection system as an aspect of quality assurance.

Four companies (in Belgium and Norway) indicated to slaughter immunocastrates, and three companies (in Belgium and Canada) expect to start slaughtering them in the next years. The median number of immunocastrates slaughtered per week was 575 at a mean slaughter line speed of 580 pigs per hour.

Two companies (in Spain and Norway) indicated that they did not slaughter nor expected to start slaughtering entire male pigs or immunocastrates in the next years.

Detection method requirements

Of the EU-based respondents 84% (26 out of 31) indicated that a boar taint detection system is needed, whereas only 25% (1 out of 4) respondents from a third country indicated this. The five EU based slaughter companies that indicated that no boar taint detection system was needed were situated in Spain and Belgium. Most slaughter companies (93% in EU, 100% in third countries) indicated that a “yes/no” scale for boar taint is sufficient and that reporting of boar taint results to the farmers is necessary (79% in EU, 100% in third countries). Of the ten respondents that had a detection system, four indicated to report the results of the boar taint assessment back to the pig producer and three indicated to not report the results back.

The most important aspects of a boar taint detection system at the slaughter line for slaughter companies were the easiness to adapt to changes in the parameters used to measure boar taint, compliance with food safety regulations, and work place safety. The
least important requirements were the mobility of the boar taint detection system, adaptation to slaughter line speed, and full automation.

Method specifications
Out of the 12 slaughter companies in Belgium, Denmark, France, Germany and the Netherlands with a boar taint detection system in spring 2013, ten provided information on the type of detection system. Nine out of the ten companies that stated what boar taint detection system was in place, had a system based on sensory analysis and one had an on instrumental analysis based system. The sensory systems were based on androstenone smell, skatole smell and off-odour. The instrumental based system could detect skatole concentration. All systems assessed all carcasses.

Methodology
For the nine slaughter companies that indicated to have a boar taint detection system based on sensory analysis, most used one assessor per carcass to determine boar taint, several used two and one used four assessors per carcass. All companies used a heating method to determine boar taint. The most used method was hot air and gas powered torch (both 3 slaughter companies), followed by electric soldering iron, oven and boiling in water (all 1). The slaughter companies with a sensory based boar taint detection system had a mean slaughter line speed of 511 pigs per hour and a mean analysis time of 5.2 seconds per carcass. The maximum time for continuous sensory assessment was between 30 to 60 minutes (median 30 minutes) and assessment took place at slaughter line speed. The median assessment time was six seconds (range 2-10). On an average day the median number of boars checked by one assessor was 130 (range 10-1,000).

The company with an instrumental method had a line speed of 360 pigs per hour and an average analysis time of 30.0 seconds per carcass.

Location and sampling location
The nine companies with a sensory method, measured boar taint in the neck and one in the loin. All used these sampling locations, because samples from these locations contain subcutaneous fat. Six companies performed the sensory methods online, two of them after meat inspection, and four just before cooling, and two slaughter houses performed the analysis elsewhere in a laboratory setting. The instrumental analysis was performed elsewhere in a laboratory setting. Almost all the slaughter companies indicated a preferred location of the boar taint detection system in the slaughter line. Most slaughter companies (18 out of 32), indicated that the preferred location for boar taint detection was located just after the meat inspection. Only two companies indicated the cooling area as a preferable location.

Training and accuracy
All companies had a selection and training procedure for sensory assessors of between 3 days and 3 weeks with at least 8 hours of training. Eight of these mentioned that assessors
should be sensitive to androstenone and to skatole. Seven also mentioned that the assessors should show satisfactory performance in a laboratory setting and five additionally demanded that they also show satisfactory performance in a slaughter line setting. All but one company evaluated assessor performance, mostly using chemical compounds and cross-validation with other assessors. The slaughter company using the spectrophotometry method, indicated to assess method performance daily.

Six companies indicated to evaluate assessor performance, only one indicated not to. The frequency varied from daily to annually. Evaluation was mainly done by chemical compounds and by cross-validation with other assessors. Only two of these companies provided efficiency and accuracy numbers.

**Costs**

The EU-based slaughter companies indicated a median acceptable maximum costs for testing for boar taint of €0.50 per tested entire male, with a spread from 0.00 € to 2.00 €. One company in a third country indicated acceptable maximum costs of 0.04 € per tested animal. Three companies provided initial investment costs of their sensory based boar taint detection system. Initial investment costs ranged from 3,600 € to 12,000 €. Seven slaughter companies provided the running costs of their system. Six of them used a sensory method and one an instrumental method. Median running costs for a sensory system were 1.50 € per slaughtered entire male pig (range from 0.20 € to 2.68 €) and 1.34 € per slaughtered entire male pig for the instrumental system.

### 4.1.3 Identification/definition of industrial method specifications

A detection system for boar taint at the slaughterline should comprise a total solution covering the registration of individual animals, analysis and integration with the IT system.

The system could take on two forms:

**Analysis in separate laboratory**

A sample is extracted from the carcass and sent to an in-house laboratory where it is analysed.

![Figure 1](image1.png)

**Figure 1.** Schematic overview of a system in which a sample is physically removed from the carcass.

The analysis part of the system overview can be performed using an instrumental technique or a human nose method.

For a system as described in **Figure 1**, the following procedures will be necessary.
The individual animal is registered based on its gambrel identification (ID). The supplier number can be linked to this ID (logistics) if the result of the measurement is to influence the payment to the farmer. At the same time, the sex of the animal is recorded. These processes are a prerequisite of, though not a part of, this requirement specification. A sample of fat from the carcass of the male pig is taken and placed in a vial. The vial containing the sample has its own ID, which is related to the gambrel ID in the database. Experience shows that it would be an advantage to use pre-marked disposable vials.

The acquired sample is assumed to be representative of the entire animal. The sampling is expected to be performed manually. The sample is taken using appropriate equipment e.g. a drill system.

Solutions that focus on both the financial and environmental costs must be chosen.

The use of disposable items minimizes the sources of error that occur (cross contamination) in connection with cleaning and also makes it possible for the sample to be sealed more efficiently with film. The use of disposable vials may well prove financially and environmentally attractive.

For an instrumental method, the sample usually has to be weighed. Having to weigh and record each individual sample has proven to be problematic in terms of process technology and is also time-consuming.

The result of the measurement must be available in time for carcasses that have been tested positive to be handled separately when leaving the chilling room. Thus, it should be possible to use the information accumulated in the databases to automatically recall positive carcasses from the cold storage for further processing or transport. At larger slaughterhouses this is typically done using Programmable Logical Controllers (PLC) for diverting carcasses from one conveyor to another.

Measurement at the slaughter line

Alternatively, the method can be implemented directly at the slaughter line without physically removing a sub-sample, e.g. by using a hot iron/hot air method directly on a selected part of the carcass.

![Figure 2. Schematic overview of a system where boar taint assessment and registration are performed directly on the slaughter line.](image)

Again it is assumed that the heated area of the carcass contains boar taint components that are representative for the entire carcass.

A system, as depicted in Figure 2, will require the following handling.

A small part of the carcass is heated directly in order to bring the boar taint components into the gas phase. It is assumed that the heating of the local area can be performed in an adequately standardized manner. The boar taint is then assessed using a “human nose”
method or by a very robust instrumental method. The result can be either stamped on to the carcass or digitally linked to the gambrel ID and entered into a database.

Such a system will in all likelihood require that the area surrounding the measurement site is purged using a fresh air supply so that odours from the surroundings do not influence the results or dull the ability of the sensory panellists to sense off-odours.

Ideally, the sample should be evaluated in the same place it is taken, thus eliminating the need for it to be transported. However, both equipment for chemical analysis and a “human nose” type measurement can be difficult to implement on the slaughter line as these measurements typically are very sensitive to the surroundings with water vapour, noise, vibrations and many ambient odours. In some cases, the costs of protecting the measurement equipment or building a protected platform for a human nose solution will exceed the costs of setting up a transport system for samples and a suitable analytical room.

The solution whereby a separate laboratory is used is advantageous in that it provides the flexibility to service several slaughter lines simultaneously. With the laboratory separated from the slaughter line, it will also be easier to have backup equipment, thus reducing the risk of shutdowns.

If it turns out that it is necessary to establish a transport system, it should be designed in such a way that it can be easily cleaned and maintained.

The analytical procedure should be automated as much as possible. The larger the slaughterhouse, the more the need for automated sample handling and analysis. When the samples have been taken, they must be placed in a buffer system before being analysed. The buffer size must be determined, taking into account production variations. It is expected that the key challenge regarding the measuring technique will be to release the boar taint components indole, skatole and androstenone and possible other boar taint related compounds from the fat samples in a fast and reproducible way.

If an instrumental method is preferred, it is likely that the androstenone assay will be more time-consuming and expensive than the assay for skatole/indole. This is due to the stronger binding to the fat of androstenone compared to skatole.

The other big challenge will be to make a system that gives sufficiently reproducible results.

In case of a chemical analysis it is preferable to use solvents that are inexpensive and do not harm the environment.

The analytical method should have a good resolution and a high reproducibility at concentrations of indole/skatole and androstenone in the fat samples close to the threshold region separating acceptable from unacceptable levels. The ability of the system to measure is assessed by comparing with measurements obtained by an established reference method which comes close to a "gold standard" (Wenzl 2014). The number of false positive and false negative measurement results must be kept at a minimum, and the threshold for the binary sorting must be chosen to balance the risk of customer complaints (too low sensitivity) against the cost of too many false positives (too low specificity). However, this balancing of risk against cost is open for negotiation between the pork industry and its customers.

To test the performance of the method it is necessary that the reference analysis is performed on almost the same sample as the method has used. As a measure of how well the equipment or sensory evaluators function in relation to a selected reference (gold
standard) method (Wenzl 2014) or a certified sensory panel, the definitions sensitivity, specificity and reproducibility can be applied. Such validation procedures must be carried out at regular intervals.

As an example of a system measuring skatole/indole and androstenone in back fat samples, the following demands could be set:

In Table 1 a set of requirements are listed that could act as guidelines for a future system operating at a large abattoir.

Detection and quantification limits in Table 1 are based on mean values and expected sorting thresholds for distinguishing positive from negative entire males (Babol et al.1996) have published results with androstenone and skatole levels in slaughter weight pigs in the ranges from 0.00 - 1.75 ug/g and 0.02 - 0.68 ug/g, respectively, with corresponding average values 0.47 ug/g and 0.20 ug/g. The applied thresholds for distinguishing negatives from positives can arguably be set at 0.26 ug/g and 0.80 ug/g.

Table 1. Suggested guidelines for an instrumental system measuring androstenone and skatole in back fat samples.

<table>
<thead>
<tr>
<th>Specification</th>
<th>Skatole</th>
<th>Androstenone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorted according to:</td>
<td>0.012 ug/g</td>
<td>0.04 ug/g</td>
</tr>
<tr>
<td>Detection limit</td>
<td>0.04 ug/g</td>
<td>0.11 ug/g</td>
</tr>
<tr>
<td>Quantification limit</td>
<td>30 min (max*)</td>
<td>High</td>
</tr>
<tr>
<td>Analytical time</td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Capacity</td>
<td>Adequate for measuring all entire males slaughtered</td>
<td>***</td>
</tr>
<tr>
<td>Accuracy</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Specificity</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

*) A buffer system where sample pre-treatment can be performed should contain as many as 150 samples depending on the time required for equilibriums to establish. Analysis time should be below 12 seconds.

**) Agreement with the assigned value of a reference standard, or with the content derived by a reference method within maximum relative uncertainty of 10 %...

***) Free from matrix or spectral interferences

For a chemical or instrumental method, the analytical time is defined as the total time from a sample has been extracted from the carcass until a result is available. Thus the analytical time includes sample extraction from the carcass, transport of sample to laboratory, time spent by each sample in the buffer system, sample pre-treatment and finally the actual analysis. Traditionally, it has been required that the analytical time should be short enough to allow for the sorting process to take place before the carcasses are transferred to the cold storage room. However, with the use of Radio-frequency identification (RFID) tags on the gambrels, an analysis result can be postponed until just before the carcass is to be processed further or shipped. This delay will, however, impair production planning as it is not known in advance, how many carcasses can be used without restrictions.

D5.2 Final report
In Table 2 an example is listed for the basic requirements for a sensory method applied at an abattoir.

**Table 2:** An example of specifications for a sensory panel sorting system.

<table>
<thead>
<tr>
<th>Specification</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorted according to</td>
<td>Boar taint</td>
</tr>
<tr>
<td>Analytical time</td>
<td>as low as 5 seconds*</td>
</tr>
<tr>
<td>Capacity</td>
<td>Adequate for measuring all entire males slaughtered</td>
</tr>
<tr>
<td>Uptime</td>
<td>High (back-up personnel required</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>90 %</td>
</tr>
<tr>
<td>Specificity</td>
<td>98 %</td>
</tr>
</tbody>
</table>

*) Strongly dependent on slaughter line speed

**) The specificity must be set much higher than the sensitivity catching only 90 % of the positives results in a few customer complaints, but a specificity of 98 % will result in the unjust downgrading of almost 2 % of all male carcasses which is very costly.

**Validation**

The measurement system, whether it is based on an instrument or a sensory panel, is validated on an ongoing basis to ensure that the functionality is intact. Procedures are drawn up that regularly test the system against reference test items with a known content of the active substances or with known odour scores. Efforts must be made to ensure that the start-up procedure for the system/method matches other processes in the abattoir in terms of time.

The measuring capacity of the system must correspond to a situation in which at least half of the pigs slaughtered at any slaughterhouse are male pigs. If this is not possible, more systems will have to run in parallel.

If the measurement fails to work, procedures must be drawn up to ensure that a new measurement can be made. When the measurement has been made and approved, the result must be recorded.

When the measurement of the levels of the boar taint is obtained, the result must be recorded in the IT system and sent to the Programmable Logical Control (PLC) for the sorting process to take place in combination with the existing procedures. It will therefore be possible to introduce a sorting process that is dependent on the odour score for each carcass or, if an instrumental method is used, dependent on the different combinations of skatole and androstenone levels.

The results are communicated via a suitable interface to the operating staff and other stakeholders, and appropriate daily reports are drawn up. Statistical data for the period are also compiled.
The system must comply with any regulatory requirements regarding measuring and labelling of products.

The following actors are considered to be potentially involved in, or have an interest in, the running of the system and could therefore have an interest in this requirement specification:

Authorities, consumers of the slaughter products, farmers, plant managers, supervisors, technical staff, sales/marketing, laboratory technicians, local and external service technicians.

Authorities
The authorities could potentially call for a ban on the castration of piglets and, although boar taint in no way possess a health risk, introduce legislation concerning labelling. Authorities might be involved, if the pork producer is paid for the pigs he delivers according to the result of the boar taint measurement. Procedures will be drawn up that describe the activities necessary for quality assurance. This will include a procedure whereby the measurement system is validated against reference units on samples with a known content of the relevant substances.

End product consumer
The system must provide sufficient data and measurement stability for the products to be labelled according to customer needs. The objective of this is to ensure that the consumer avoids, as far as possible, unpleasant odours and flavours when cooking pork cuts from male pigs which could turn them from buying pork.

Meat processing and allocation of meat for different products
The system must provide sufficient data and measurement stability so that, with regard to the sorting process, the products can be used for the most profitable purposes.

The pig breeder
Procedures must be drawn up that make it possible to pay the pork producer according to the optimal potential value of the animals supplied. The payment system can be used to encourage the producers to produce pigs with less boar taint.

Plant manager and supervisors at the abattoir
The system and the measurements must make it possible to plan and use the slaughter material for the most profitable purposes and provide customers with high quality products.

Technical staff, service and laboratory technicians at the abattoir
The system must be robust and operationally reliable. The operation of the system must be easily manageable and have clear built-in indicators and alarms in case of operating errors.

Sales and marketing departments at the abattoir
The measuring procedure must make it possible to use the slaughtered animals for the most profitable purposes and provide customers with high quality products.
The system must provide a high degree of assurance that the quality of the fresh meat and processed products gives the customers an experience that encourages them to continue buying pork products.

In addition, some basic measurement requirements need to be fulfilled.

**What must be measured?**
The level of boar taint in a representative part of each entire male carcass.

**Sensitivity and Specificity**
The threshold for discriminating acceptable from unacceptable (negative from positive) carcasses may be set in such a way that the prospects of receiving customer complaints (low sensitivity) are balanced against the risk of downgrading too many untainted carcasses (low specificity).

In case of a chemical measurement of the three boar taint components, the detection limits must lie well below the desired sorting thresholds for skatole/indole (approx. 0.25 ug/g) and androstenone (0.50 - 1 ug/g). The combined results for both skatole and androstenone will result in altered sorting limits, and the desire for a more varied sorting process (e.g. grading on a 5 point scale) for different purposes will add to demands for the accuracy of the measurements.

If an on-site sensory evaluation is used, reproducibility between different assessors must be ensured on an adequate level and that the analytical results do not “drift” from day to day.

**Measurement resolution and accuracy**
The resolution of the measurements must be such that it is possible with a predefined level of certainty to distinguish between boar taint levels in samples with skatole/indole concentrations differing by 0.03 ug/g and androstenone levels differing by 0.06 ug/g, otherwise demands for either sensitivity or specificity will have to be relaxed considerably.

**Reproducibility**
The outcome of a classification (positive or negative) of repeated measurements, on the same homogenized fat samples by independent operators at different points in time, should be the same within certain predefined limits. This type of method evaluation should be performed on samples with boar taint levels close to values discriminating acceptable from unacceptable.

**Capacity**
The capacity of the system must ensure that all boars handled at an abattoir are analysed. If all male pigs are un-castrated, the system must handle half of the hourly slaughtering. If 600 pigs are handled per hour, the system must produce a valid measurement every 12 seconds on average. If male pigs are slaughtered in batches, the system must produce a result every 6 seconds.

For an instrumental method it should be noted that the sample pre-treatment e.g. homogenization, addition of solvents, reagents and heating can be done in a buffering system or in an auto sampler. Such a design can leave ample time for establishing the necessary equilibriums between sample and headspace or between sample and solvent.
Time required to obtain a result
Fast response time is in general important. However, demands for a short response time (minutes) depend on the work procedures at individual abattoirs. For an instrumental method, a buffer system could be built, and the time spent in it by the sample could be used for sample pre-treatment and for the establishment of the desired equilibriums. An autosampler system could ensure that samples are treated in exactly the same way and for the same duration before analysis.

Stability
The system must be robust and operationally reliable ensuring that maintenance costs are minimal.

Properties of the reference method
The fundamental reference method should be calibrated to consumer acceptance via an assessment of androstenone and skatole/indole levels or other chemical components that may prove important. Otherwise, long term stability will be very difficult to maintain, and large differences in perception between countries can be expected. The thresholds for consumer acceptability must then be set by relating the reference measurements to assessments made by a sensory panel under controlled conditions. The reference method should be reproducible with an SE between repeated measurements on the same sample that is at least five times below the sorting thresholds chosen for both skatole and androstenone.

System size and design
Most abattoirs are characterised by having very limited space available for new installations. Extensions to the slaughterhouse buildings and making rearrangements to the slaughter line are very costly. Therefore, a boar taint analysis system should take up limited amounts of space.

Materials
Materials that come into contact with the carcasses must comply with regulations on food contact materials.

Approvals. Compliance with legislation concerning food safety, labour conditions and environment
The system must comply with existing national and EU legislation on food safety, labour conditions and environment.

Set-up costs
The greatest costs of setting up a system are related to the necessary structural alterations, setting-up of a system for collecting and transporting of samples, purchase of equipment and training. The solution should generally be as simplistic as possible and be based on commercially available instrumentation.
Operating costs
Based on Danish experience, acceptable operating costs are in the order of 1.3 Euro per measurement including payment to laboratory staff. However, the figure may well vary from one country to another.

Servicing, operational lifetime and maintenance
The system must be robust, operationally reliable and easily maintained.

Operation
The system must be easy to operate, and the interface must be designed in such a way as to avoid faulty operation.

System delivery and data format
The system must be robust and operationally reliable.

Operational lifetime
The system must be robust and operationally reliable. Procedures must be drawn up for scheduled replacement of wear parts.
Feasibility and cost assessment of methods used or being developed in pig slaughter plants in the EU (WP2)

Critical review of technical performance and feasibility of existing and potential rapid methods

Method performance selection criteria

The results from Work Package 1 were used to establish a template of method performance criteria to be used for further selection and prioritization of relevant methods for rapid sorting of boar tainted carcasses at the slaughter line.

A critical issue is the boar taint reference standard ("gold standard") that is used for determining the accuracy of the assessment methods. Ideally, the gold standard should be the consumer perception of boar taint, but this is very difficult to achieve. The most commonly used approach is to use the levels of the compounds responsible for boar taint (androstenone and skatole). This has the advantage of being objective measurements, but their relationships with the consumer perception of boar taint are still somewhat controversial and there is no agreement on the threshold levels that should be used for sorting. Other parameters, such as sensory assessment by expert panels (or by an extremely good expert), could also be used as gold standard for boar taint, provided that there are sufficiently related to consumer perception of boar taint. Due to various possible industrial scenarios with regard to sorting, rather a range of the method performance parameters has been suggested (Tables 3-5).

Table 3. Performance criteria for instrumental substance specific methods.

<table>
<thead>
<tr>
<th>Method parameters</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods</td>
<td>1 method</td>
</tr>
<tr>
<td>Accuracy</td>
<td>i</td>
</tr>
<tr>
<td>Precision</td>
<td>≤ 10 %</td>
</tr>
<tr>
<td>Specificity</td>
<td>Free from matrix or spectral interferences</td>
</tr>
<tr>
<td>Limit of quantification (LOQ)</td>
<td></td>
</tr>
<tr>
<td>LOQ in fat phase</td>
<td>0.05-0.10 µg/g S^ii, 0.10-0.25 µg/g A^iii</td>
</tr>
<tr>
<td>LOQ measurement on carcass/adipose tissue</td>
<td>0.025-0.05 µg/g S^ii, 0.05-0.12 µg/g A^iii</td>
</tr>
<tr>
<td>Method capacity</td>
<td></td>
</tr>
<tr>
<td>Samples analysed pr hr</td>
<td>100-800 carcasses</td>
</tr>
<tr>
<td>Analysis speed per sample</td>
<td>4 – 40 sec</td>
</tr>
<tr>
<td>Sampling time per sample</td>
<td>&lt;20 min</td>
</tr>
<tr>
<td>Result reporting</td>
<td></td>
</tr>
<tr>
<td>Off-line method</td>
<td>&lt; 30 min</td>
</tr>
<tr>
<td>On-line method</td>
<td>&lt; 1 min</td>
</tr>
<tr>
<td>Costs</td>
<td></td>
</tr>
<tr>
<td>Running cost per carcass</td>
<td>&lt; 2.0 Euro</td>
</tr>
</tbody>
</table>

^i agreement with the assigned value of a reference standard, or with the content derived by a reference method within maximum relative uncertainty of 10 %. ^ii The degree of agreement between independent measurements. The precision is set to 10 % with regard to the LOQ to account for a measurement uncertainty +/-10 % that assures correct classification of positive or negative sample at the sorting criteria levels. ^iii Calculations for LOQ are based on the following suggested lowest limits for threshold levels : 0.20 µg/g for skatole and 1.0 µg/g for androstenone on fat basis.
Table 4. Performance criteria for instrumental fingerprinting methods.

<table>
<thead>
<tr>
<th>Method parameters</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods</td>
<td>1 method</td>
</tr>
<tr>
<td>Accuracy</td>
<td>1</td>
</tr>
<tr>
<td>Precision</td>
<td>≤ 10 %</td>
</tr>
<tr>
<td>Specificity</td>
<td>Free from matrix or spectral interferences</td>
</tr>
<tr>
<td>Precision</td>
<td>≤ 10 %</td>
</tr>
<tr>
<td>Limit of quantification</td>
<td>LOQ in fat phase 0.05-0.10 µg/g S&lt;sup&gt;i&lt;/sup&gt;, 0.10-0.25 µg/g A&lt;sup&gt;ii&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>LOQ measurement on carcass/adipose tissue 0.025-0.05 µg/g S&lt;sup&gt;iii&lt;/sup&gt;, 0.05-0.12 µg/g A&lt;sup&gt;iii&lt;/sup&gt;</td>
</tr>
<tr>
<td>Method capacity</td>
<td></td>
</tr>
<tr>
<td>Samples analysed pr hr</td>
<td>100-800 carcasses</td>
</tr>
<tr>
<td>Analysis speed pr sample</td>
<td>4 – 40 sec</td>
</tr>
<tr>
<td>Sampling time pr sample</td>
<td>&lt;20 min</td>
</tr>
<tr>
<td>Result reporting</td>
<td></td>
</tr>
<tr>
<td>Off-line method</td>
<td>&lt; 30 min</td>
</tr>
<tr>
<td>On-line method</td>
<td>&lt; 1 min</td>
</tr>
<tr>
<td>Costs</td>
<td></td>
</tr>
<tr>
<td>Running cost pr carcass</td>
<td>&lt; 2.0 Euro</td>
</tr>
</tbody>
</table>

<sup>i</sup> agreement with the assigned value of a reference standard, or with the content derived by a reference method within maximum relative uncertainty of 10 %.<sup>ii</sup> The degree of agreement between independent measurements. The precision is set to 10 % with regard to the LOQ to account for a measurement uncertainty +/- 10 % that assures correct classification of positive or negative sample at the sorting criteria levels. <sup>iii</sup> Calculations for LOQ are based on the following suggested lowest limits for threshold levels: 0.20 µg/g for skatole and 1.0 µg/g for androstenone on fat basis.

Table 5. Performance criteria for sensory/human nose methods

<table>
<thead>
<tr>
<th>Method parameters</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods</td>
<td>1 method</td>
</tr>
<tr>
<td>Accuracy</td>
<td></td>
</tr>
<tr>
<td>Sensitivity&lt;sup&gt;i&lt;/sup&gt;</td>
<td>90-100 %</td>
</tr>
<tr>
<td>Specificity&lt;sup&gt;i&lt;/sup&gt;</td>
<td>95-100 %</td>
</tr>
<tr>
<td>Precision</td>
<td>≤ 10 %</td>
</tr>
<tr>
<td>Method capacity</td>
<td></td>
</tr>
<tr>
<td>Capacity/analysis pr hr</td>
<td>100-800 carcasses</td>
</tr>
<tr>
<td>Analysis speed pr sample</td>
<td>4 – 40 sec</td>
</tr>
<tr>
<td>Sampling time pr sample</td>
<td>0.5-20 min.</td>
</tr>
<tr>
<td>Result reporting</td>
<td></td>
</tr>
<tr>
<td>Off-line method</td>
<td>&lt; 30 min</td>
</tr>
<tr>
<td>On-line method</td>
<td>&lt; 1 min</td>
</tr>
<tr>
<td>Costs</td>
<td></td>
</tr>
<tr>
<td>Running cost pr carcass</td>
<td>&lt; 2.0 Euro</td>
</tr>
</tbody>
</table>

<sup>i</sup> % ability to identify positive samples as determined by a golden standard method. <sup>ii</sup> % ability to identify negative samples as determined by a golden standard method.

Other method selection criteria:
In addition, other practical industrial and DG-SANCO method requirements shown in Table 6 (Contract Annex II) need to be fulfilled, which are still rather unspecific, but which we feel are mostly covered by our suggestions Tables above).
Table 6. Requirements according to DG SANCO contract (Annex II).

<table>
<thead>
<tr>
<th>DG-SANCO method requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection methods</td>
</tr>
<tr>
<td>Capacity/ Analysis speed</td>
</tr>
<tr>
<td>Sensitivity</td>
</tr>
<tr>
<td>Reproducibility</td>
</tr>
<tr>
<td>Accuracy</td>
</tr>
<tr>
<td>Methods</td>
</tr>
<tr>
<td>Accuracy – correct classification rate</td>
</tr>
</tbody>
</table>

‘in entire and immunocastrated male pigs.

Depending on the customers’ perception of the risk associated with boar taint, and on the possibility to use tainted meat in processed products, various sorting threshold levels will need to be applied, which will require also various method demands with regard to sensitivity and specificity. To meet the various needs, a range of the different method performance parameters have been applied for the method selection criteria to be able to match also various industrial scenarios of detection systems and performance requirements. As example, a 95 % sensitivity would mean that there will be 5 % false negatives. Further, the required sensitivity will depend on the slaughter plants sorting criteria with regard to levels of skatole and androstenone, so therefore it is appropriate to rather use a sensitivity and specificity range for the method selection criteria. The same would also apply to the cut-off levels of the boar taint substances, suggesting rather a concentration range as methods performance criterion.

Whether indole should be included as well is still in discussion, but then sorting and sensory threshold levels should be established and should also relate to sensory perception levels. For the skatole equivalent method being used in Denmark, the sensory impact factor, i.e., contribution to sensory perceived boar taint, is respectively 0.75 for skatole and 0.25 for indole (Danish data, Claus Borggaard).

It is also essential to relate the golden standard method performance and the sorting thresholds to consumer acceptance data.

Investment and running costs have not been included here as a method selection criterion. Costs are further considered in section 3.2.2 of this report and section 3.2.3 integrates the performance and cost criteria.

Methods being used at slaughter plants in the European Union

Human nose methodology

During the method survey carried out during WP1, nine European slaughter companies were identified who have a detection system based on sensory assessment and which was applied to assess all carcasses.

Most of these used one assessor per carcass to determine boar taint, several used two and one used four assessors per carcass. In all methods all entire males were assessed at the normal slaughter line speed by heating subcutaneous fat.
The sensory systems were based on androstenone smell, skatole smell and off-odour. Some companies performed boar taint analysis directly on the carcass (on-line methods), and four were conducting the analyses off-line (at-line methods). Most sensory systems were located in the slaughter line; the exact location differed between the slaughter companies. The maximum time for continuous sensory assessment was between 30 to 60 minutes and assessment took place at slaughter line speed. The capacity ranged from 350 to 680 carcasses per hour (mean 511/h). Depending on the number of assessors used, this corresponds to an analysis time from 8 to 2 seconds (mean 5.2 sec) per carcass. For offline use, sampling would add more minutes to the analysis time of this method. Total analysis time per carcass varied from 2 to 10 minutes when the assessment was conducted on- and at-line. These numbers fall within the method capacity requirements (Table 6). On an average day the median number of boars checked by one assessor was 130 (range 10-1 000).

Two respondents of the industry method survey (D1.2 report, Current situation in EU and third countries spring 2013) reported performance: The rate of false positives ranged from 2-20 % (11 % median) and false negatives from 2-40 % (21 % median). These high false rates would have dramatic economic consequences for the pig meat industry. Accordingly, there is a need for further method development to improve the performance with regard to accuracy. Further, the precision between assessors was reported to be within 2 %, which is satisfactory.

So far, only one study at industrial level with a great sample material has been scientifically published (Mathur et al, 2012). The study was carried out on sample material from 6.574 male pig carcasses from the slaughter line at VION group, The Netherlands. However, the sensory assessment was performed in a laboratory setting. It should also be mentioned that these results were not based on a scoring system with yes-no as alternatives, but with a scoring system using a five point scale from 0 to 4. Nine persons were trained on detecting boar odor and used for this study. The method performance, on individual assessor level, needed improvement with regard to reproducibility and accuracy. The correlation with androstenone varied from $r=0.2-0.5$ and skatole varied from $r=0.3-0.9$. Further the average reproducibility weighted for the number of samples between assessors was 23 %.

Information regarding accuracy of the human nose method is scarce. Data regarding (online) method performance with chemical analysis and expert panels as reference method are needed. These tests should be performed in several slaughter companies as practices between these companies differ, such as selection and training of experts, slaughter line speed, sample location and heating method. Several of these can probably be optimized to improve the method.

The experiences so far from the European industrial trials using the sensory based human nose methodology have also addressed the need for further method development regarding both sampling and selection and training of assessors. The performance of these methods with regard to industrial method specifications still need to be properly validated.

In order to further prove whether this human nose method strategy may be a future solution at industrial level for sorting boar tainted carcasses, it is recommended that this methodology is prioritized as one of the methods to be further tested and validated as intended during WP4 of the project, to verify whether the industrial detection system requirement can be fulfilled. This would also be in line with the European pig slaughter companies view, which observe that the human nose methodology is the only one that is
currently available for an industrial use, in the absence of any viable instrumental method. The view of the scientific community is that the measurement uncertainty of sensory methods is high, particularly in the conditions of the human nose methodology. If instrumental methods in future can replace the sensory assessment at the slaughter line, this would most likely be a better solution on long term.

**Instrumental methods**

During the method survey carried out during Work Package 1, it was documented that only one instrumental detection system is in use for sorting boar tainted carcasses, which was the Danish colorimetric method that is applied to assess all carcasses. This is so far, the most successful on-line method that has been taken into use on an industrial scale for the purpose of sorting boar-tainted carcasses. The colorimetric method measures the sum of both skatole and indole (Mortensen and Sørensen, 1984). The colouring reagent does not discriminate between indolic compounds so the method is not specific for skatole. Results are therefore reported as “skatole equivalents” since both indolic compounds are detected at the same wavelength. A back fat sample is physically removed from the carcass, followed by solvent extraction, addition of colouring reagent and spectrophotometric fluorescence measurement of skatole and indole. The method was introduced in Danish slaughter plants from 1991 (Hagdrup 2009). Interestingly, the results obtained by the colorimetric method were in good agreement with sensory assessments of boar taint by an expert panel (r=0.8, Hansen-Møller, 1998). Up to 360 samples per hour can be tested, and the analytical error of the method is reported to be 0.04 ppm on fat basis (Hansen-Møller, 1998). However, the limitation of this technology is that androstenone cannot be measured, and therefore with regard to the industrial application, an additional detection system for androstenone will be needed. The Danish experience at industrial level with this methodology has been hampered by relatively high maintenance costs and a complicated troubleshooting, and it has been difficult to maintain personnel and expert knowledge (Hagdrup 2009). Accordingly, there is currently no dedicated on- or at-line instrumental measurement system available for sorting of boar tainted carcasses that measures both androstenone and skatole.

**Potential methods in development**

**Instrumental methods**

The results from the method survey carried out during Work Package (Task 1.1, Survey of methods used and in development and 1.2, Current situation of rapid detection methods in slaughter plants) have shown that there are several methods in development, and that some of them may have a future potential for rapid sorting of boar tainted carcasses at the slaughter line.

**Spectrophotometry**

The gas-phase measurement that combines Fourier Transform Infrared (FTIR) with Photo Acoustic Spectroscopy (PAS) (Kauppinen et al., 2004), is a very fast technique, with a potential for on-line use. It has been shown that the boar taint compounds have distinguishable gas-phase IR spectra that would enable direct detection in the vapour phase (Haugen et al., 2008; 2009). These methods do not comply with the very low limit of
detection that is required for measuring the boar taint substances in the gas phase. However, the analytical sensitivity may be enhanced by extending the measurement time. This would, however, be on the expense of sample capacity by increasing the analysis time.

In addition, gas sampling is a critical issue that still needs to be optimised and adapted to slaughter line conditions, and which would be the time limiting factor in the total analysis time.

The same FTIR-PAS technology has also been applied to the solid phase; in a recent feasibility study, the measurements of boar fat with levels of androstenone ranging from 0.28-0.5 ppm, have shown that it is possible to discriminate the different levels of androstenone (Haugen et al., 2009). However, more research and development needs to be carried out on this technology to document its further suitability for industrial use.

Gas-phase fingerprinting/Gas-sensor array

Since the boar taint compounds occur at very low concentrations in the gas-phase and these technologies have an insufficient selectivity, they are not able to measure the boar taint substances specifically, and therefore strongly rely on frequent calibration against known levels of the skatole and androstenone, or sensory assessment of boar taint. This requires multivariate statistics for the data handling of the sensor readings and reference data (levels of substances or sensory). Consequently, the accuracy and precision of this technology will be hampered by the measurement uncertainty of the reference data to which it has been calibrated.

The published research in this field comprises limited laboratory feasibility studies analysing pure lipid phases (oils and fats) spiked with androstenone or skatole and mixtures of both compounds at different concentration levels, as well as real back fat samples from boars with different levels of skatole and androstenone. The results show significant correlation (r=0.6 to 0.9) between the sensor readings, levels of skatole and androstenone, and sensory attributes related to boar odour and flavour. The achieved correct classification rates with regard to cut-off levels of skatole and androstenone or sensory data vary from 60 to 90 %, i.e. too high rates of respectively false positives and negatives.

So far the existing literature in the field presents limited feasibility studies carried out at laboratory level with a limited number of samples and lacks proper validation and documentation of performance parameters. It is therefore essential for further work in this field, that these techniques are being validated properly to prove their fitness for purpose.

In addition, there are still technical issues related to the performance of gas sensors, which limit their application. The suitability of these systems is highly dependent on the required operation conditions of the sensors used. The typical issues of this technology are still their poor reproducibility, sensor recovery, sensor drift, sensor replacement and need for recalibration and lack of robustness to an external environment (humidity and temperature variations).

It must be emphasized also, that existing commercial systems “off the shelf” will not be suitable for the boar taint application since further application research and development must be considered to end up with a dedicated system for this purpose.

To improve the selectivity of this methodology, recent research and development has combined separation techniques like gas chromatography with gas-sensing devices, using
gas-sensors as GC detectors. These micro-machined GCs’ allow the detection of single volatile compounds within 10-60 seconds. In particular, uncoated surface acoustic wave (SAW) sensors have been used for this purpose (Bodenhöfer et al., 1996). The use of this technology in combination with gas enrichment techniques could also have a potential for future on-line detection of boar taint. But this would require further research and development.

Various gas-sensor array systems (Electronic nose) are commercially available with different gas-sensor technologies and including also integrated headspace sampling systems. These systems represent purchase costs ranging from 10 000 to 40 000 Euro.

**Mass-spectrometry**

The Mass Spectrometry (MS) based methods have the advantage that they fulfill the industrial requirements with regard to selectivity, limit of quantification (LOQ) and precision (Table 3), measuring the individual boar taint substances indole, skatole and androstenone specifically. However, most of the MS-based methods identified during the method survey of WP1 combine chromatographic separation technology, and require additional sample extraction/cleanup ahead of the instrumental analysis, which add significantly to the analysis time, resulting in too long analysis times, and therefore will not be able to match the industrial analysis capacity requirements. In case of analysis in the gas phase, these aspects can be exemplified by the recently developed method by Fischer et al (2011) using automated headspace solid phase micro extraction combined with gas chromatography (HS-SPME-GC/MS) for the simultaneous quantitation of the three boar taint substances skatole, indole and androstenone in pig fat. In addition, also two androstenol isomers can be quantitated with this method. The method starts with methanolic extraction of melted fat followed by freezing and evaporation for enrichment of the analytes. Since the headspace sampling (30 min. at 100 C) and desorption (20 min.) into the GC take in total 50 minutes, and the GC run lasts for about 30 minutes, the analysis capacity of the method being only about one hour per sample.

In case solvent extraction of fat is combined with GC/MS methodology, also a tedious and time consuming cleanup step is required ahead of the instrumental analysis, that would increase the analysis time and thereby limit the measurement capacity. Even by applying automated sample cleanup procedures by using robotics, this method strategy would not allow the high analysis capacity needs of the industry.

So even fully automated detection systems based on this methodology would require that several detection systems are applied including the necessary technical personnel.

The same limitations also apply to liquid chromatography based methodology. They fulfil the method performance criteria with regard to selectivity, LOQ and precision. And by the use of Ultra Performance Liquid Chromatography (uPLC) separation techniques, analysis time can be reduced down to 6 minutes. But the need for sample clean up procedures increases the total analysis time. When high resolution mass spectrometry is used as detection technique, this represents also a sophisticated and very costly technique that would require high level of technical personnel to operate. (Bekaert et al., 2012).

The recently investigated Proton Transfer Reaction (PTR) technology in combination with high resolution Time of Flight Mass spectrometry (Borggaard et al., 2012) based on direct gas-phase analysis without using a chromatographic separation and which is still under
development, has shown to have limitations with regard to fulfil the requirements to LOQ of androstenone (Table 3). Besides, the method represents also a rather sophisticated and very costly technology based on Time Of Flight Mass Spectrometry (TOF-MS) and would require high level of technical personnel to operate.

Mass Spectrometry has also been applied to measure boar taint indirectly by combining pyrolysis with direct mass spectrometry (Berdagué et al. 1996; Ampuero and Bee, 2006, 2008). This technique is based on pre-heating and partly combustion of a fat sample in a pyrolysis chamber and the vapour phase is transferred directly into the ion-source of the mass spectrometer, followed by mass fragmentation of the molecules. Since all the volatile compounds are fragmented, the output data represent the accumulated fragment masses over the scanned mass range, and therefore chemometrics is required to interpret the data. This method represents a fingerprinting method, since it has not the high selectivity of the above described methods. It can be combined with different sampling methods and the most commonly used are headspace or pyrolysis. Results have shown that high classification rates (Ampuero and Bee, 2008) can be obtained with regard to cut-off levels of respectively skatole and androstenone. However there remain still some technical challenges and further applied research and development is required to consider this technique to be suitable for slaughter line conditions. Issues include the technical operation of the system, sampling, frequent calibration, data analysis and system maintenance related to contamination of both the transfer line from the pyrolyer to the MS and the ion source of the MS-system.

Another recently developed mass spectrometry based technology is the Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) directly applicable to gas-phase analysis. Very few other alternative methods offer such a simple technology to operate, and which have a high selectivity and short analysis speed (less than 1 minute per sample). In addition, it is a very selective technique with detection limits down to parts per trillion (ppt) levels of volatile compounds in the gas phase. This technology has already been applied to real time gas monitoring applications, and can be operated on-line continuously and automatically. A feasibility study has been carried out by the manufacturer of these systems (www.syft.com), which has proved that it is possible to detect indole, skatole and androstenone at low levels in the gas phase. However, further testing of this methodology would be needed to validate its performance with regard to the industrial method requirements (Table 1), and it has to be adapted to a standardized sampling system.

Another recent technique which may have a potential as boar taint detection system is the Rapid Fire Mass Spectrometry (RF-MS) system concept of Agilent. The system can be integrated either with a low resolution quadrupole based MS for Liquid Chromatography -/MS/MS analysis or a high resolution Time of Flight (TOF) MS system. The system has a high analysis capacity due it’s fast analysis time, i.e., 5-12 seconds per sample. But the sampling step still will need to be developed and adapted to industrial requirements. The low resolution system is a by far simpler technique than the high resolution TOF-MS system, which is a very costly technology (TOF-MS) and would require high level of technical personnel to operate.

The MS-technologies and combined GC/MS systems described represent purchase costs ranging from 100.000 (low resolution) to 600.000 (high resolution) Euros.
Immunological methods

The immunological methods apply to the measurement of androstenone only, since immunological assays for skatole have not yet been successful. The method complies with the sensitivity and specificity requirements (Table 1), but the major weakness of this methodology with regard to method performance requirements is the analysis time. The time limiting step of these methods is the incubation time for the reaction to take place between the analyte (acting as antigen) and a complementary antibody, which may vary from 30 to 60 minutes. In addition, an initial extraction step is required ahead of the incubation step, which adds to the analysis time, and the final washing and detection step of the method amounting to a total analysis time of 1-2.5 hours. Another issue that applies for some assays is related to selectivity, i.e. the cross-reactivity of the antibody towards other steroids than androstenone present in the sample. This methodology represents also a number of steps of manual work so for industrial use, it would require that all the method steps are fully automated.

Despite the fact that there have been some method improvements recently (Patent No. 9, Deliverable report D1.1) still the total analysis time is around 60 minutes. By applying 96 well plates, 40 samples can be analysed in parallel, i.e. 40 samples an hour. But this number could be increased by running several assays in parallel.

Insect-based biosensors

Recent work has shown that insect based biosensing may have a potential for boar taint detection (Wäckers et al, 2011). It was demonstrated that the wasps could detect low, medium and high concentrations of indole, skatole and androstenone directly in boar fat at room temperature, and the levels tested were around the sensory thresholds levels for these compounds (Olson et al, 2012). Time needed for the wasps to learn the odours are 3 minutes, and detection time varied from 10 to 40 seconds. A US patent application of this measurement principle has been submitted (Patent No. 10, D1.1 report).

A simple prototype detector system exists, but still the gas sampling has to be standardised and adapted to the detector system. However, there are a few issues that need to be considered for the practical application at industrial level: The use of insects in the slaughter line to detect boar taint may be in conflict with food safety legislation, but since they are kept in a closed detector box, there will be no risk for free flying insects in the slaughter environment as long as the insects are managed properly. In addition, the wasps have a limited life time, i.e. about 7 weeks that would require a facility and personnel at the slaughter plant for rearing, keeping and training the insects before use. The costs of this methodology compared to other instrumental detection system are very low (500-3 000 Euros). There is a potential for automation of both the management and training procedures so that it can be envisaged to use the methodology on-line. But there is still a need for developing the method to adapt it to the slaughter line.

The use of sniffer pigs instead of insects for the detection of boar taint has been investigated (Aluwé and Bekaert 2009). Although the performance of the sniffer pigs was reasonably good, the uptake of this detection method by the industry has little promise, because the training of the pigs was slow and labour-intensive. Moreover, the use of animals in the slaughter line to detect boar taint can be in conflict with food safety legislation, which prohibits the presence of animals in a slaughter plant.
**Electrochemical sensors**

Electrochemical sensors are currently under development at UWE (Crew et al., 2009). They can be used for simultaneous measurement of both skatole and androstenone, and if necessary also indole and other compounds related to boar taint. The principle of electrochemical sensors for skatole and androstenone analyses has been protected by a UK patent application (D1.1 report, Method survey, Patent No. 11, UK1212727, University of the West of England, Bristol). So far, the prototype sensors have been developed under laboratory conditions and tested on standards solutions of skatole and androstenone and using meat cuts. Current limit of detection is 0.25 ppm for androstenone and 0.01 ppm for skatole. Measurement time takes approximately 30 seconds and total analysis time is suggested to be one minute, but which has not yet been documented. The method allows for measurements to be conducted directly on the carcass or meat cuts, by direct insertion of the sensors in any area of the carcass or meat cut. The sensors can be disposable, which prevents cross-contamination and does not require washing-up step. The sensors can also be incorporated in existing abattoir setting and integrated in the central carcass/meat quality analysing system if necessary. Results can be displayed immediately after the measurement either as “Yes” or “No” options for boar taint or as exact concentrations of skatole, androstenone and other boar taint compounds (if required). However, this technology has not been evaluated under real industrial setting yet and no commercial prototype is available. It would require more development work to adapt the method to slaughter line conditions, and a proper validation of the method with regard to industrial method requirements still needs to be conducted before it can be implemented at industrial level.

**4.2.2 Cost assessment of implementation and development of existing and potential rapid methods**

**Sorting strategies**

When estimating boar taint by measuring the boar taint components skatole-indole and androstenone separately, it is possible for the slaughterhouse to allocate their raw materials to specific uses according to the individual levels of skatole/indole and androstenone.

The importance of androstenone and skatole respectively is product dependent. For instance studies including Danish flank roll, bacon and smoked, cooked ham were performed. Androstenone, skatole and indole were analysed on neck fat samples using HPLC with fluorescence detection. In Danish flank roll, androstenone and skatole have a major effect on perceived boar taint, and, to avoid boar taint, the androstenone content should be below 2.1 ppm (if the skatole < 0.05 ppm), and the skatole content should be below 0.3 ppm (if the androstenone < 0.2 ppm). In smoked streaky bacon, androstenone and skatole have a major effect on perceived boar taint, and androstenone is solely responsible for boar taint during cooking and should be below 0.9 ppm to avoid boar taint. Boar taint-related sensory attributes in smoked, cooked ham were strongly affected by serving temperature and androstenone, but no effects of skatole, indole and core temperature were observed. Pungent odour/flavour, sweaty odour and urine odour were the sensory attributes most affected by androstenone and serving temperature. Therefore, in order to optimize the use
of meat from entire males it is valuable when measuring boar taint to analyse all three boar taint flavour compounds - skatole, indole and androstenone.

A simple example could be that a slaughterhouse sorts the carcasses into 3 levels of androstenone (low, medium and high) and 2 levels of skatole/indole (low and high) as shown in Table 7.

With this choice the carcasses could be grouped into 6 classes each suited for use in one or more specific types of product. A carcass with both low skatole and low androstenone would be an all-purpose raw material that qualifies for being served as a warm roast or cutlet whereas products type 5 could only be served as cold pork, e.g. cured products.

**Table 7. Suggested carcass classes.**

<table>
<thead>
<tr>
<th>Sorting classes</th>
<th>High Skatole</th>
<th>Low Skatole</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Androstenone</td>
<td>products type 5</td>
<td>products type 2</td>
</tr>
<tr>
<td>Medium Androstenone</td>
<td>products type 4+5</td>
<td>products type 1+2</td>
</tr>
<tr>
<td>Low Androstenone</td>
<td>products type 3</td>
<td>all products</td>
</tr>
</tbody>
</table>

If Indole is included in the sorting process the above table will be a cube or the indole content can be added to the skatole level with some predefined factor.

One option could be to base a sorting threshold on skatole equivalents (Danish colorimetric method):

\[
skatole\text{ equivalents} = 0.75 \cdot [skatole] + 0.25 \cdot [indole]
\]

The actual values of the weighted importance of skatole and indole (0.75 and 0.25 in the above equation) must be determined experimentally.

**Sorting based on continuous versus discrete scales**

For the sorting strategy the major difference between using an instrumental solution instead of a human nose solution can be summed up as:

- The human nose method results in discrete classes, e.g. high-low or high – intermediate – low depending on the number of classes that can be resolved by the method.
- The instrumental methods result in continuous scales for the individual boar taint components even though each measurement is associated with a confidence limit.

This means that for the sensory method two carcasses that have very similar contents of boar taint components may fall into separate sorting groups. With an instrumental result the same two carcasses can be recognised as being quite similar.

This is graphically shown in Figure 3 where a number of back fat samples have been measured for their contents of skatole and androstenone. The red lines represent chosen thresholds for the two components. The 4 samples within the blue circle will with a high – low discrimination as with a sensory method be classified as belonging to 4 very different classes. However, with an instrumental method these 4 samples can be recognized as being...
almost similar if the precision of the measurements are sufficiently good. This will make it possible to apply more intelligent sorting strategies resulting in less loss due to carcasses being classified false negative or false positive.

Figure 3. Back fat samples sorted for their contents of skatole and androstenone. Red lines represent the chosen thresholds.

Consequences for the industry

If a total stop of surgical castration of male pigs is implemented throughout Europe, meat from carcasses found to contain unsatisfactory high levels of odoriferous compounds will not be suited for warm servings or for exports to many countries outside the EU. This means that alternative uses will have to be found for this type of meat. At the moment, entire male pigs are slaughtered on a small scale in a number of EU countries so until now it has not been a major problem to find these alternative uses for tainted meat. However this may very well change if the use of surgical castration ceases altogether. In some countries a deduction is made in the payment to farmers for each tainted carcass they deliver to the slaughterhouse.

In Denmark where entire males have been slaughtered on a small scale (approximately 3% of the entire male pig population) farmers are deducted 22 € for un-castrated male pigs with unacceptably high levels of skatole and indole. In a situation where this practice is scaled up to cover the entire pig production throughout Europe (annually 250 million pigs, source Eurostat) it may be very difficult to find a market for all downgraded male pigs.

This extrapolation is a stylized approach to illustrate the economic aspects. We do realize that it is a simplification of the Danish case. The question is how in the new market equilibrium benefits and costs are allocated - via the price mechanism - to the various supply chain segments. Boar tainted carcasses have a lower market value. How much lower depends on the total number of boar tainted carcasses that enter the EU market, which is the product of the number of entire male pigs and the average boar taint prevalence in the EU. This latter percentage will be influenced by the extent to which farmers use preventive measures.
Calculations for operational costs

In the following calculations the term *labour requirements* (LR) will be defined as the number of persons continuously involved in the process for operating the boar taint detection system. If possible it is assumed that the people involved in the boar taint detection can be set to perform other tasks when spare time is allowed.

**Sensory method**

*At-line sensory solution*

Calculations for the sensory method are based on the assumptions:

- Each sample is assessed in the laboratory by 2 persons independently of each other. If the two panellists do not agree the sample will be classified as “maybe” indicating that the sample is on the boundary between acceptable and unacceptable. Furthermore a barcode reader is used to automatically enter the sample ID on the beaker containing the sample into the slaughterhouse computer system.
- It is anticipated that each panellist requires 27 seconds for performing one assay. This amount of time allows the panellist to purge her nose so as to prevent excessive carry-over from the last sample, press a button for delivering a yes/no answer and for removal of the sample.
- When running at full line speed the panellists will have to be serviced by at least one person conditioning the sample, presenting the samples to the panellists and removing samples after evaluation.
- At each slaughter line one person is working with sample extraction and if the labelling and dispatch is not automated one more person will be necessary for performing this task. The staff requirements for this task are guided by the operators having to keep up with slaughter line speed.

Equation 1 is used to calculate the labour requirement (in the following denoted LR) as a function of the number of assays to be performed each day. Inside the parentheses the first term is the number of assessors required in the at-line laboratory performing the assay (in duplicate, therefore the factor 2) and the second term the number of people working on the slaughter line collecting samples and dispatching. The last terms compensate for holidays and sick days.

\[
\text{LR} = 2 \cdot \left( \frac{\text{assays}}{\text{day}} \cdot \frac{\text{sec}}{\text{assay}} \cdot f + \frac{\text{samples}}{\text{day}} \cdot \frac{\text{sec}}{\text{sample}} \right) \cdot \frac{51 \text{ weeks}}{\text{effective weeks}}
\]

Where the term effective weeks are calculated as:

\[
\text{effective weeks} = (51 - \text{weeks holidays}) \ast (100\% - \% \text{ sick days})/100\%
\]

The sec/sample term is set so the slaughter line operators can keep up with the line speed and can be calculated as 3600 sec/hour divided by the number of boar carcasses per hour. If the slaughter line speed is 400 then 3600 sec/200 boars leaves 18 seconds per sample for sample extraction from the carcass, labelling and dispatching to the at-line laboratory.
The factor f is a servicing factor in the range 1-2 representing the person necessary to present samples to the panellists and remove samples after the assessment. If this factor is not included (or set to 1) the performance of the panellists will have to be adjusted downwards accordingly.

Example:

For a slaughterhouse killing 90,000 pigs per week, half of which are male pigs, there will be a need for performing 9,000 assays per day (5 days a week). A line speed set at 400 pigs per hour (200 male pigs) results in there being 18 sec per sample for taking the sample from the carcass and dispatching it to the laboratory. Daily work hours per person are set to 7.5 h (=27,000 sec/shift).

Furthermore we assume that each of the two panellists require 27 seconds/sample. The servicing factor in the sensory laboratory is set at \( f = 1.25 \) corresponding to one service staff for every two panellists.

With the number of sick days/person = 5% and weeks holiday/year = 5, the labour requirement according to equation 1 is 40 persons.

Notice that equation 1 takes into account that panellists can perform other functions when they are not needed in the laboratory. However, it can be disputed whether the two panellists can be set to performing tasks directly on the production line as the ambient air should not be allowed to dull their ability to judge the presence of boar taint components.

The total number of persons involved in the sensory analysis will have to be much greater than the number given by equation 1. If, for example, an assessor can perform 200 evaluations per shift then with the above example 90 people will have to be rotated in and out of the sensory panel during a day (2 shifts). This means that more than half of the personal working directly with the slaughtering will have to be part time allocated to the boar taint sensing. This is of course not possible as no slaughterhouse has people employed that have 20% of their time to spare for odd jobs.

On-line sensory solution

If it is possible to perform reliable measurements in the slaughter line environment a work platform for the sensory panel can be built so samples can be assessed directly on the carcass. By doing this the two persons working in the laboratory can be moved to the slaughter line thereby eliminating the need for sample extraction and transport of samples to an at-line laboratory.

If boar taint is assessed using the so called hot-iron method it is assumed that two persons are necessary to evaluate each carcass and that the evaluations are done independently of each other.

Equation 1 now simplifies to:

\[
LR = 2 \cdot \left( \frac{\text{samples}}{\text{day}} \right) \cdot \left( \frac{\text{sec}}{\text{sample}} \right) \cdot \frac{51 \text{ weeks}}{\text{effective weeks}}
\]

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Example:
At 9 000 assays per day, a cadence of 18 seconds per sample and a shift duration of 7.5 hours = 27 000 seconds \( \Rightarrow LR = 12 \) persons.

It is seen that the on-line method is a factor 3-4 less demanding than the at-line sensory method when it comes to staff requirements.

Again, if the persons involved in the boar taint measurements are to be rotated to other tasks after 200 assays each, then 60 persons (1/3 of the slaughter line work force) will have to be involved in the boar taint detection business.

**On-line sensory solution, current practices.**

At a number of slaughterhouses throughout Europe, where entire male pigs are slaughtered, boar taint is assessed on the slaughter line directly from the carcass. The most commonly used method is the so called hot iron method. A single operator is used for warming a small section of the back fat and subsequently sensing any malodour due to boar taint coming from that carcass. The operator then enters the result of her evaluation directly into the slaughterhouse computer system by pressing a button.

The labour requirements for this scenario is according to equation 3:

\[
Eq. 3: \quad LR = \left( \frac{\text{samples}}{\text{day}} \right) \times \left( \frac{\text{sec}}{\text{sample}} \right) \times \left( \frac{\text{sec}}{\text{shift}} \right) \times \left( \frac{51 \text{ weeks}}{\text{effective weeks}} \right)
\]

Example:
The cadence is governed by the requirement that the operator(s) must be able to keep up with the normal line speed. If the line speed is set at 400 pigs per hour. The sampling time will be equal to 18 sec/sample. With 9000 samples (entire males) per day and the number of effective weeks per year equal to 43 (51 weeks – holidays and sick days) the labour requirement will be: \( LR = 7 \) persons.

However, if a person can be expected to perform 200 samples in a single work shift then in the above example 22-23 persons per shift (1/3 of the slaughter line workforce) will have to be job rotated in and out of the boar taint evaluation task.

**Instrumental method**
The instrumental solution can be implemented in one of two basic ways.

- A fully automated at-line method where samples are acquired from each carcass and then sent to an in-house laboratory room where the sensitive instrumentation can be protected from the harsh environment at the slaughter line.
- The instrumentation can be placed within a protective casing on the slaughter line where the equipment is safe from water splash, detergents and ambient odours.
**Fully automatic at-line solution**

Assumptions:

- 10 seconds per assay on each instrument.
- At each slaughter line one person working with removing samples from each carcass and putting each sample in a vial.
- Automatic labelling of vials containing the sample.
- Automatic weighing of the samples.
- Automated sample dispatch from slaughter line to laboratory.
- 1 person can man the laboratory if the number of assays is kept below 2000 per day, otherwise 2 will be needed. As with the sensory method it is assumed that spare time can be used for other purposes.
- Above 5000 male carcasses per day a second instrument must be installed to run in parallel with the first instrument. The analysis time per sample will thus be halved.

With these assumptions equation 4 is used for calculating labour requirements for a fully automated instrumental method. The first term in the parentheses is the fraction of a day required to analyse all back fat samples for that one day (if this fraction exceeds 1 it means that the laboratory will have to work in 2 shifts). The second term is identical with the corresponding term in equation 1 (sensory method) and describes the time spent by the operator on the slaughter line.

Eq.4: \[ LR = \left( \frac{\text{assays}}{\text{day}} \times \frac{\text{sec}}{\text{assay}} \times \frac{3600}{\text{sec}} \times \frac{7.5}{\text{day}} \right) \times \frac{\text{assays}}{\text{day}} \times \frac{\text{sec}}{\text{shift}} \times \frac{51 \text{ weeks}}{\text{effective weeks}} + \frac{\text{No lab staff}}{1} \]

**Example:**

Input parameters are here the same as used in the previous section for the at-line sensory method.

Assays/day: 9 000 (e.g. three lines working in two shifts 400 carcasses per hour).

Sick days: 5%, weeks holiday: 5.

Analysis time (instrumental time): 5 sec/assay (10 seconds on each of the parallel running instruments).

No of lab staff: 2 persons.

Sampling rate of the operator working at the slaughter line: 18 seconds/sample.

Result: LR = 11 persons
**Fully automatic on-line solution**

If there is space available at the slaughter line an analytical instrument could be built into a box with protection rating IP 67 (submersible in water for up to 30 minutes) or better and placed directly adjacent to the carcass sampling station. Such a solution will make the automatic sample dispatch system and the laboratory personnel redundant. The inside of the protective compartment could be flushed continuously with odour free, dry air so the instrumentation can be protected from the harmful environment.

The instrumentation could be based on e.g. Gas-phase fingerprinting/Gas-Sensor Array, Mass Spectrometry or Ion Molecule Reactions together with the appropriate automated sample pretreatment in auto samplers for feeding the analyzer. At this stage it is not possible to make a trustworthy assessment of an on-line instrumental method as there are too many unknowns that need resolving. However, if this solution turns out to be viable the costs of operation will be lower than for any of the above mentioned scenarios.

Under the most favourable conditions the labour requirements for running the system directly on the slaughter line would be given by the equation 5:

\[
LR = \frac{\text{assays}}{\text{day}} \cdot \frac{\text{sec}}{\text{sample}} \cdot \frac{51 \text{ weeks}}{\text{effective weeks}}
\]

Additionally a person per shift will have to be allocated to the task of checking the instruments.

**Example:**

With 9 000 assays per day, 7.5 hour shifts, 18 seconds per sample (line speed 400 carcasses per hour half of which are boars running) the labour requirement of the on-line instrumental method will be 8.4 persons. However, this will require separate instruments at each slaughter line, in this case 3.

**Comparing instrumental and sensory based systems**

In Figure 5 are shown the labour requirements for methodologies, based on equations 1 and 3 for the at-line sensory and the at-line instrumental systems respectively.
In Figure 4 the discontinuities in the staff requirements for the instrumental method are due to one additional person in the laboratory when more than 2000 assays are handled per day and to the deployment of an additional instrument when more than 5000 assays are performed per day thus doubling the throughput.

From figure 2 it is seen that for performing 1000 sensory based assays a day the time consumption per assay is of the order 0.005 days (7.5 h) corresponding to a labour cost of 0.9 € per assay which is in good agreement with the running costs identified from the questionnaire replies received from the slaughterhouses as reported in the D1.2 report of this project. And this is also consistent with the analysis cost assessments from the ALCASDE project, which was 1.1 € per pig carcass (ALCASDE final report, 2009)

Installation costs

The costs for training the personnel are not included in the following calculations of the installation costs for the sensory systems and the instrumental systems. Even though many more people will be involved in the boar taint detection if an at-line sensory panel is performed, the instrumental laboratory solution will require highly trained laboratory technicians. In both cases the training of personnel will not contribute greatly to the overall costs for implementing the method.

The following two scenarios are evaluated:

1. Sensory panel solution.
   - A sensory laboratory close to the slaughterlines equipped with necessary facilities and a ventilation system providing odour neutral air. Adaptation costs for the sensory laboratory are estimated to be in the range 70,000 € to 160,000 € depending on the number of staff.
- A Work platform at each slaughterline for extracting samples from the carcasses. Cost is set at 20,000 € per slaughterline.
  The number of slaughterlines is set at the integer part of the ratio between assays per day and 3,000 boars per day corresponding to working one line in two shifts at line speed 400 carcasses per hour.

2. Instrumental solution.
- An instrumental solution based on mass spectrometry. The price per unit is set at 370,000 €. Above 5,000 assays per day two instruments will be necessary.
- An autosampler capable of handling an adequate number of samples and maintaining a constant temperature used to feed each MS unit is set at 60,000 € per unit.
- Labeling system. When fat samples are placed in the vials at the slaughterline a bar code containing the carcass ID must be attached to each vial. Estimated cost per unit: 5,000 €. This part can be omitted and replaced by a bar code reader if disposable vials with preprinted bar codes are used.
- Weighing station and sample dispatch station (both automated). In most cases the samples will have to be weighed. This can be done at the work platform on the slaughterline. The transport system can be built as a pneumatic dispatch system. Vials are passed to the laboratory room in a pipe system driven by pressurised air. The cost of this system is set at 80,000 € per slaughterline.
- The number of slaughterlines is set at the integer part of the ratio between assays per day and 3,000 boars per day corresponding to working one line in two shifts at line speed 400 carcasses per hour.

\[ \text{Installation costs} \]

![Installation costs graph](image)

**Figure 5.** Expected installation costs for the two different scenarios, at-line sensory panel and at-line automated Mass spectroscopic solutions.

With the above assumptions the installation costs can be determined as a function of the number of assays per day for the two different scenarios. This is illustrated in **Figure 5**.
**Maintenance**

The two scenarios, sensory and instrumental systems will both require regular maintenance. In the case of a sensory system the performance of each panellist will have to be checked at regular intervals. For this purpose fat standards will have to be available for testing the sensitivity and repeatability of the staff. In case of one or more panellists deviating from the rest a recalibration will be necessary. One way of ensuring that the performance of the panellists remains constant is to insert known samples (including blanks) at regular intervals during the day, e.g. every 20th sample. If the assessments made by one of the panellist starts to drift that person will have to be retrained.

In the case of an instrumental solution, system performance will have to be checked at regular intervals. This will also be done using fat standards with known concentrations of androstenone, skatole and indole. Typically the system will need checking at the start of each shift. The weighing unit and the temperature regulation of auto sampler will need checking as will the analytical instrument e.g. the mass spectrometer.

However, as the maintenance costs in the two scenarios are of the same order of complexity they have been neglected in the following cost estimations.

**Time to breakeven**

It is now possible to estimate whether it is profitable to invest in an at-line fully automated solution compared to choosing an at-line sensory analysis (human nose).

The profitability can be calculated as a payback time for one’s investment. In this case the payback time is calculated as the difference in initial investment between the two methods divided by the difference in cost of operation (yearly wages for operators/panellists).

Eq.6:

\[
\text{Time to breakeven (years)} = \frac{\text{Initial cost instrumental method} - \text{Initial cost sensory method}}{\text{yearly operating cost (sensory - Instrumental)}
\]

The result of applying equation 6 is presented in Figure 6. Here it can be seen that the time to breakeven decreases with the number of assays per day.

The abrupt change at 5 000 assays per day is due to it being necessary to acquire an additional mass spectrometer unit including its auxiliary equipment when more boars are slaughtered.
Figure 6. Payback time for additional investment in a fully automatic at-line instrumental method compared with an at-line sensory method.

It must be emphasised that the calculation of the payback time does not take into account the indirect costs associated with carcasses being classified as either false positives or false negatives.

**Cost of developing a system for large scale industrial conditions.**

Currently there are both sensory and instrumental based methods capable of measuring boar taint under controlled laboratory conditions, and in some countries boars are already slaughtered on a smaller scale where such laboratory procedures are applied. Sensory based systems are already in use on an industrial scale at slaughterhouses in certain EU countries. However, both the sensory (human nose) and the automated instrumental systems need to be thoroughly validated while working under large scale production conditions.

**Instrumental method**

Before a system for measuring androstenone can be installed at abattoirs performing routine on-line analysis the analytical instrument needs to be paired with appropriate auto-samplers and extraction systems. Rapid mass spectroscopy systems are available as off-the-shelf products. Manufactures of mass spectrometers can deliver systems capable of rapidly measuring parts per billion (ppb) concentrations in solutions and MS systems sampling directly from headspace in concentrations down to ppt (ng/kg) levels (the instruments act as an advanced electronic nose identifying individual chemical components). Some of these instruments are capable of performing an assay in less than 10 seconds (see references). If these instrument types are to be used at slaughterhouse line speeds it will be necessary to work with the sample presentation prior to measurement, involving the entire range of steps from the sample is extracted from the carcass until the pre-treated subsample can be injected into the spectrometer, be it based on any of the suggested methods identified in the method survey of this project (D1.1 report, Method survey).
The automatic pre-treatment steps may involve:

- Weighing of samples
- Automatic barcode reading.
- Homogenization or melting of fat sample.
- Addition of solvent
- Holding samples at constant temperature during pre-treatment.

All of these steps can be performed in auto-sampler arrangements of which numerous solutions are commercially available (see references).

It would be preferable that the sample taken at the slaughter line is placed directly in barcode labelled vials by the operator. A barcode reader can automatically link the barcode of each vial to the ID of the carcass from which the sample originates. This can be done prior to the vial being dispatched to the laboratory for pre-treatment and subsequent chemical analysis.

It is therefore advisable that a concerted R&D effort be undertaken to bring the potential rapid instrumental methods for measuring androstenone to a level where they can be brought on-line. Also the human nose methods need to be validated under full slaughter line speed thereby making it possible to test its potential.

All of these steps that may be necessary for an automated instrumental solution have been developed at the Danish Meat Research Institute back in the 1980’ies. This system has been in operation at a large Danish slaughterhouse since 1991. However, numerous of-the-shelf products of this type are now commercially available from a range of providers.

A number of different instrumental methods have been proposed in this project (WP1, tTsk 1.1, Survey of methods in use and in development and WP2 Task 2.1, Critical review of technical performance and feasibility of existing and potential rapid methods). In any case it will be necessary to have a large quantity of reference back fat samples with known levels of boar taint and concentrations of its main odour components androstenone, skatole and indole that can be used for calibrating and testing the on-line systems or sensory panels, 500 reference samples would most likely be needed during development. These samples can be in the form of larger blocks of fat that can be subdivided into smaller sample sizes necessary for testing the instrumentation.

Development costs for a fully automated boar taint system (best estimate) are 3.000.000 €.

This figure should cover leasing/buying instrumentation for the project. Reference analysis in sensory laboratory and chemical analysis for individual boar taint components.

Sensory (human nose) method

As demonstrated by the above cost estimates it is likely that sensory methods will be preferred at smaller slaughterhouses with less than 1000 slaughtered boars per day. Moreover, in the absence of viable instrumental method, such methods are currently used at industry levels in a number of large slaughter plants in Europe.

This method does not require a large investment for developing instrumentation. However, the reproducibility, sensitivity and selectivity must be assessed under prolonged industrial of operation at normal line speeds.

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The following issues need attention:

- How many samples can one assessor evaluate per day with the required levels of reproducibility, sensitivity and selectivity?
- Does the ability to sense boar taint deteriorate during the day?
- What is the repeatability and what is the reproducibility between assessors?
- What are the day-to-day variations of each assessor and can biases between assessors be kept under control during routine operation?
- How do ambient conditions affect the measurements? Can reliable measurements be made with panellists working directly next to the slaughter process?
- What is the most efficient and reproducible method for heating the sample for boar taint assessment (hot iron, gas torch, hot water method, microwave oven etc.)?

The trials for answering these questions are estimated to cost 1.500.000 €.
4.2.3 Integrated method evaluation

Evaluation of sensitivity and specificity

For a binary classification system, sensitivity (capacity to identify tainted carcasses) and specificity (capacity to identify non-tainted carcasses) of an assessment method must be evaluated against a golden standard method which tells whether a carcass is really tainted or untainted.

<table>
<thead>
<tr>
<th>Assessment method</th>
<th>Golden standard method</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Assessed as untainted</td>
<td>Really untainted</td>
<td>Really tainted</td>
</tr>
<tr>
<td>AU_RU</td>
<td>AU_RT</td>
<td>AU = AU_RU + AU_RT</td>
</tr>
<tr>
<td>AT_RU</td>
<td>AT_RT</td>
<td>AT = AT_RU + AT_RT</td>
</tr>
<tr>
<td>RU = AU_RU + AT_RU</td>
<td>RT = AU_RT + AT_RT</td>
<td></td>
</tr>
</tbody>
</table>

AU: Assessed as Untainted, AT: Assessed as Tainted, RU: Really Untainted, RT: Really Tainted

Sensitivity = AT_RT / RT ; False negative = AU_RT / RT
Specificity = AU_RU / RU ; False positive = AT_RU / RU

The critical issue is the golden standard method to be used.

Ideally, the golden standard should be the CONSUMER perception of boar taint, but this is impossible to achieve.

The most commonly used golden standard is the levels of the compounds responsible for boar taint (androstenone and skatole) as measured in reference to an analytical standard method. This has the advantage of being objective measurements, but their relationships with the consumer perception of boar taint are still somewhat controversial and there is no agreement on the threshold levels that should be used for sorting tainted meat. The DG SANCO supported study (CAMPIG, Study on consumer acceptance in the European Union and in third countries of pig meat obtained from male pigs not surgically castrated) on consumer acceptance of boar taint has investigated the consumer perception and acceptability of boar tainted meat. However, this study was not able to conclude on single acceptance threshold values for androstenone and skatole.

Other parameters, such as sensory assessment by expert panels (or by an extremely good expert), could also be used as golden standard for boar taint, provided that the relationship of this parameter to the consumer perception of boar taint is documented and turns out to be good enough.

Evaluation of costs

Operational costs have been evaluated (see section 3.2.2) for various methods for the example of a plant in a Denmark slaughtering 90,000 pigs per week and operating with 3 lines (400 carcasses per hour each) and 2 shifts per day, the operational costs (mostly labour costs) would be
- Around 0.2 € per tested carcass for an on-line method with two sniffers
- Around 1.0 € per tested carcass for an at-line method with two sniffers.
- Around 0.20 € per tested carcass, to which possible costs for chemicals and reagents should be added, for a fully automated on-line instrumental method.
- Around 0.25 € per tested carcass, to which possible costs for chemicals and reagents should be added, for a fully automated at-line instrumental method.

Because operational costs are mostly made of labour costs, they will vary according to countries, according to the levels of wages.

Operational costs per carcass
- do not vary much according to the number of carcasses tested for sensory methods
- decrease with increasing number of carcass tested for instrumental methods

These operational costs do not include the costs of maintenance:
- performance checking and retraining of sniffers in the case of sensory methods
- performance checking and maintenance of instruments for instrumental methods. Performance checking is expected to be less costly for instrumental than for sensory methods.

These operational costs also do not include the indirect costs associated with misclassified carcasses either false negatives or false positives.

**Table 8. Summary of operational and investment costs in the case of a plant in Denmark slaughtering 90,000 pigs per week (3 lines and 2 shifts per day).**

<table>
<thead>
<tr>
<th></th>
<th>On-line sensory</th>
<th>At-line sensory</th>
<th>On-line instrumental (1)</th>
<th>At-line instrumental (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operational costs, € per tested carcass (2)</td>
<td>0.2</td>
<td>1.0</td>
<td>0.2 (4)</td>
<td>0.25 (4)</td>
</tr>
<tr>
<td>Investment costs, € per tested carcass (3)</td>
<td>0</td>
<td>0.02</td>
<td>0.09</td>
<td>0.12</td>
</tr>
<tr>
<td>Total costs, € per tested carcass (2, 4)</td>
<td>0.2</td>
<td>1.0</td>
<td>0.3</td>
<td>0.37</td>
</tr>
</tbody>
</table>

(1) Mass spectrometer
(2) Costs do not include maintenance and indirect costs associated with misclassified carcasses.
(3) Amortisation on 5 years, i.e. 11.5 million tested carcasses
(4) Do not include costs of chemicals and reagents.

**Investments costs** have also been evaluated (see section 3.2.2) for the same example as above:
- Very low for an on-line sensory method.
- In the range of 200,000 € for an at-line sensory method (sensory lab, sampling platforms)
- In the range of 1.0 million € for an on-line instrumental method using mass spectrometry. These costs could however be augmented due to the necessity of protecting the instrument against the harsh conditions of the slaughter line.
- In the range of 1.4 million € for an at-line instrumental method using mass spectrometry

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The costs for the studied example (90,000 pigs per week) are summarized in Table 8. Smaller facilities would experience similar costs for sensory methods where investment costs are low. They would experience higher costs for instrumental methods where investment costs are much higher.

Sensory methods: Human nose

Compliance of sensory method according to the established method requirements (see section 3.3.2) are summarized in Table 9.

Table 9. Summary of compliance of the sensory methods to the method performance requirements.

<table>
<thead>
<tr>
<th>Method parameters</th>
<th>Requirements</th>
<th>At-line (1) Hot water (3)</th>
<th>At-line Dry heating (4)</th>
<th>On-line (2) Dry heating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods</td>
<td>1 method</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td>Accuracy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitivity</td>
<td>90-100 %</td>
<td>ND</td>
<td>☹️ (6)</td>
<td>ND</td>
</tr>
<tr>
<td>Specificity</td>
<td>95-100 %</td>
<td>ND</td>
<td>☹️ (6)</td>
<td>ND</td>
</tr>
<tr>
<td>Precision/reproducibility</td>
<td>&lt;10 %</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Method capacity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capacity/analysis pr hr</td>
<td>100-800</td>
<td>☹️</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td>Analysis speed pr sample</td>
<td>4 – 40 sec</td>
<td>☹️</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td>Sampling time per sample</td>
<td>0.5-20 min.</td>
<td>☹️</td>
<td>OK</td>
<td>OK</td>
</tr>
<tr>
<td>Result reporting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At-line method</td>
<td>&lt; 30 min</td>
<td>OK</td>
<td>OK</td>
<td>-</td>
</tr>
<tr>
<td>On-line method</td>
<td>&lt; 1 min</td>
<td>-</td>
<td>-</td>
<td>OK</td>
</tr>
<tr>
<td>Robustness and maintenance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costs (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running cost per carcass</td>
<td>&lt; 2.0 Euro</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
</tr>
</tbody>
</table>

ND: not documented or poorly documented
☹️: Not satisfactory compared to requirements

(1): At-line: Sample is taken on the carcass and transferred to a laboratory where it is assessed by sniffers in controlled conditions.
(2): On-line: Tissue is heated and sniffed directly on the carcass.
(3): Hot water: the sample is soaked for 2 minutes in a flask containing hot water and sniffed at the opening of the flask (Meinert et al., 2011)
(4): Dry heating: the sample (or the tissue on the carcass) is heated for a few seconds (soldering iron, hot air, ...) and sniffed immediately.
(5): Costs do not include chemicals, reagents and maintenance.
(6) Documented in only one scientific paper (Mathur et al., 2012); Androstenone and skatole levels as reference.

- The at-line hot water method is too slow to accommodate the speed and number of pigs. It can be envisaged however as a confirmation method to be used on the carcasses that have been identified as tainted by another method.

The at-line and on-line sensory methods using dry heating complies with requirements regarding capacity, result reporting and cost. However, the sensitivity and specificity of these
methods against a recognized golden standard is poorly documented. The scarce evidence does not allow concluding on the performance of sensory evaluation.

**Instrumental methods**

The results from the method performance and feasibility review of part 1 have been summarised in Table 10 below.

The Danish method measuring skatole equivalents is the only instrumental method that has been used at industrial level so far. The fact that this method does not measure androstenone disqualifies it for the measurement of boar taint. Moreover, its maximum throughput of 200 samples per hour is not compatible with most of the larger European slaughter plants under the hypothesis that no male pig is surgically castrated. Further the method does not comply to the requirement to analysis speed, and thereby would not qualify for having an on-line potential. In addition, the Danish experience at industrial level with this methodology has been hampered by relatively high maintenance costs and a complicated troubleshooting, and it has been difficult to maintain personnel and expert knowledge (Hagdrup 2009).

The Fourier Transform InfraRed (FTIR) based technology, applied to gas phase and solid (fat) phase would still need further research and development work to document its performance and validity with regard to the method requirements. And it would not comply to the on-line requirements of maximum one minute.

The gas-sensor array fingerprinting technologies have been proved so far to be unreliable for the detection of boar taint. Most instrumental technologies based for the detection of boar taint are based on the measurement of the concentrations of the responsible compounds (androstenone, skatole, indole). This technology fails on several critical method parameter requirements as sensitivity, specificity and on-line performance.

Since the gas and liquid chromatographic based techniques rely on chromatography combined with mass spectrometric detection, the time for the separation step adds to the analysis time, but could be reduced down to minutes, and still comply to a result reporting within 30 minutes. However, to meet the sample capacity, this would require a number of instruments run in parallel.

Further, the gas-phase based mass spectrometry techniques (SIFT-MS, PTR-MS, RF-MS) seem to fulfil most of the method criteria, but still lack a proper validation for this application before it can be document that the necessary industrial requirements are met, and more research and development work would be needed to adapt the technology to slaughterhouse conditions.

The high resolution TOF-MS systems would probably be far too costly and complex to allow for use at industrial level.

Immunological methods, which are applicable to androstenone only, would require one additional method for skatole, and would therefore not comply to the single method requirement. Besides, the method cannot comply to the method capacity and result reporting requirements.
Table 10. Summary of compliance of the instrumental methods to the requirements defined.

<table>
<thead>
<tr>
<th>Method parameters</th>
<th>Requirement</th>
<th>Spectrophotometry</th>
<th>Gas-sensor array</th>
<th>Mass Spectrometry</th>
<th>Immuno-logical</th>
<th>Sensors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Colorimetric</td>
<td>FTIR</td>
<td>GC, LC</td>
<td>SIFT, PTR</td>
<td>Insects</td>
</tr>
<tr>
<td>Methods</td>
<td>1 method</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td>ND</td>
</tr>
<tr>
<td>Limit of quantification (LOQ)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skatole</td>
<td>OK</td>
<td>ND</td>
<td>ND</td>
<td>OK</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Androsten.</td>
<td>(1)</td>
<td>OK</td>
<td>ND</td>
<td>ND</td>
<td>OK</td>
<td>ND</td>
</tr>
<tr>
<td>Accuracy</td>
<td>(2)</td>
<td>OK</td>
<td>ND</td>
<td>OK</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precision</td>
<td>&lt;10 %</td>
<td>OK</td>
<td>ND</td>
<td>OK</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Method capacity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capacity/analysis pr hr</td>
<td>100-800</td>
<td>OK-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Analysis speed pr sample</td>
<td>4 – 40 sec</td>
<td>OK-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sampling time pr sample</td>
<td>0.5-20 min.</td>
<td>OK-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Result reporting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At-line method (3)</td>
<td>&lt; 30 min</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td>ND</td>
</tr>
<tr>
<td>On-line method (4)</td>
<td>&lt; 1 min</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td>OK</td>
<td>ND</td>
</tr>
<tr>
<td>Robustness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costs (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running cost pr carcass</td>
<td>&lt; 2.0 Euro</td>
<td>OK</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not documented or poorly documented; ☺: Not satisfactory compared to requirements

(1): Agreement with the assigned value of a reference standard, or with the content derived by a reference method within maximum relative uncertainty of 10 %.

(2): Free from matrix or spectral interferences

(3): At-line: Sample is taken on the carcass and transferred to a laboratory where it is assessed by sniffers in controlled conditions.

(4): On-line: Sample is taken directly on the carcass and analyzed directly at the sorting band.

(5): Costs do not include chemicals, reagents and maintenance.

D5.2 Final report
The insect-based biosensor method is interesting, and seems to have a potential to fulfil the industrial method requirements. However, since they are still in the research and development stage, more documentation will be needed to prove their performance with regard to the industrial method requirements.

The electrochemical sensor method also demonstrated potential under laboratory conditions (including testing on real meat samples). However, further study is needed to evaluate this method against the gold standard and consumers’ perception as well investigate the options for linking these sensors to abattoir conditions.

For all the methods sampling is a critical issue, and that would limit the analysis capacity, and since this part of the analysis is not yet well documented and would need further research and development, this parameter assessment represents some uncertainty, so the table 6 entries for the parameter sampling time are uncertain.

According to the information that is available to us, there is currently no instrumental method that can be used at industry level for the detection of boar taint. Those which have been identified as promising in part 1 include:

- MS-based techniques: SIFT-MS, RF-MS, and PTR-MS;
- Insect-based biosensors;
- Electrochemical sensors.

All these methods are still at the laboratory level:

- Their limits of quantification are low enough to accommodate the envisaged sensory thresholds for androstenone and skatole.
- The results obtained so far at laboratory conditions are compatible with the requirements for capacity and time for reporting as presented in table 1. This remains however to be established in industrial conditions.
- The sensitivity and specificity have not been documented in industrial conditions.
- The most challenging issue is that they work on gas samples, except for the electrochemical sensor. The compounds have therefore to be transferred in a representative way from the fat/carcass sample to a gas sample, usually using head space techniques.

It has to be emphasized that also other relevant new emerging technologies may show up in near future, which have to be taken into consideration for this application.

It was recommended that MS based technologies (SIFT-MS and RF-MS), insect-based biosensors and electrochemical sensors be further tested against androstenone and skatole levels in real carcass samples and validated for performance in work package 4 in order to determine their sensitivity and specificity at laboratory conditions.

In addition, it was also recommended that any other method currently under development, including those for which there is no freely available information, can also be tested within WP4.

In the literature concerning the measurement of boar taint, a majority of papers support the opinion that it is the presence of the components androstenone, skatole and indole in the back fat and pork that are the main cause of the undesirable off-odour. This means that the task of documenting the reliability of a measuring system
can be greatly simplified by measuring how a potential slaughterhouse system performs compared to a chemical analysis for these three components.

A number of possible solutions for measuring boar taint have been proposed. Some of these have been partially implemented at slaughterhouses for a number of years. Of the instrumental methods, only a single fully automated system has proven its robustness by running under industrial conditions for many years. This system is designed to measure skatole and indole.

To this date there are a number of proposed rapid instrumental methods that need testing both in the laboratory and on-line at an abattoir for the rapid detection of all three components skatole, indole and androstenone. For larger slaughterhouses an instrumental method is preferable to use instead of a sensory based solution. This is due to the fact that the cost of wages for running a sensory based system will grow almost proportionally with the number of male pigs slaughtered per day. With an automated system of measurement, the operational costs are less dependent of the number of male pigs that are slaughtered per day.

There are currently no automated solutions available that have been tested adequately on a larger industrial scale with respect to reproducibility, long term stability, robustness to slaughter house environment for measuring androstenone.

For developing such a fully automated system the costs for research and development are expected to be in the vicinity of 3.000.000€ whereas the costs for resolving all outstanding questions related to the use of a sensory human nose method is expected to be around 1.500 000€.

For a slaughterhouse investing in the implementation of a boar taint measurement system the point of breakeven between using a sensory system and an automated system is expected to be in the vicinity of 2.000 male pigs per day. Above this figure the fully automated system will be economically advantageous with the extra initial investment for this solution being paid-back over the first 2.5 to 3 years of operations. These figures are derived from the comparison of two extreme methods, one sensory method requiring very high levels of labour and one instrumental method requiring very high level of investment.

However, the high demands for manning at-line sensory method used in this study are governed by the demands for maintaining acceptable levels of reproducibility, sensitivity and specificity.

Points of breakeven (in terms of number of pigs per day and years for pay-back) may vary quite extensively depending on the level of labour required by sensory methods and the level of investment required in instrumental methods.

The initial startup/installation costs are very different for a sensory based system and an automated system. In both cases the cost of establishing a work platform on the slaughter line where samples are to be taken from the carcasses, a delivery system to the laboratory, the costs for consumables together with necessary data base access are quite equal. Therefore such aspects have been neglected in this cost estimation.

The consumables (vials for samples) have also not been included in the calculations as they also are considered similar for the two scenarios.
Additionally the costs of operations given in this work do not take into account the number of misclassified carcasses either false negatives or false positives. Carcasses measured as containing unacceptable levels of boar taint that in fact are acceptable (false positives) will be a great burden for the pork industry. The opposite where carcasses containing unacceptable levels of boar taint are judged as being acceptable (false negatives) will result in loss of consumer confidence in the pork industry.

4.3 Workshop on rapid methods for use in the slaughter plants (WP3)

4.3.1 Organisation of the Workshop

An international workshop on rapid detection methods for boar taint for the pig industry and research communities was organised on the 4th of December of 2013 in the auditorium of the Institut de Recerca i Tecnologia Agroalimentaries-IRTA (Monells, Spain). During the previous days (2nd and 3rd of December) a meeting of the EAAP Working group on the Production and Utilization of Meat from Entire Male Pigs was also held in the same facilities to allow people to combine the two events.

An invitation letter, together with the preliminary program was distributed in September 2013. A reduction on the registration fee was offered to the companies who participated in the survey conducted in WP1 of the BoarCheck project.

A total of 87 companies were invited of which 35 received an invitation with reduced registration fee. These companies were distributed in 12 countries (Belgium, Canada, Germany, Denmark, France, Ireland, Italy, Netherlands, Norway, New Zealand, Spain and United Kingdom). Twelve multinationals were also invited.

With regard to the type of companies, the invitation letter was sent to 70 meat industries (abattoirs and cutting plants) and 18 instrumentation companies.

Members of the European Association of Animal Production (EAAP) working on the Production and Utilization of Meat from Entire Male Pigs (this network consists of more than 150 researchers) were informed about the two events and could therefore participate in the BoarCheck workshop.

A total of 42 participants from 8 countries (Belgium, Denmark, France, Germany, The Netherlands, Norway, Slovenia and Spain) attended the meeting. Annex 4 shows the detailed list of participants. The following stakeholders were represented:

- 27 academic research
- 4 chain stakeholders at farm level and above (breeders, feed industry, vets,...)
- 7 chain stakeholders below the farm level (slaughterhouse, processing industry, retailers, consumers...)
- 2 engineers
- 1 press
- 1 administration
During the registration, all the participants were required to sign the confidentiality agreement.

There was a presentation of the objectives of the workshop (Annex 6.1) and introduction to the project (Annex 6.2). During these two presentations a special focus was done on the confidential agreement terms.

Next, Dr. Ge Backus presented the methods with potential application to the industry and Dr. B.E. Nielsen presented technical and economic aspects and requirements of the methods. Regarding the sensory methods in the latter presentation, the authors chose to consider only those in which the sensory evaluation is performed in duplicate by two persons working independently of each other in an odour free environment. This view was challenged by some of the audience on the ground that this was not representative of today’s industrial reality.

4.3.2 Group discussions

Before the participants were distributed into small discussion groups, Dr. J.E. Haugen explained the objective of the discussion and explained terms such as method performance criteria, sensitivity and specificity, running costs, among others. After this explanation, participants were set up in groups in order to discuss 1-Sensory methods and 2- instrumental methods. Specific topics were addressed to be discussed and conclusions were recorded by each group chair person, which were presented in plenum.

Sensory discussion group: 19 participants discussed about sensory methods with potential application in the pig industry. They were distributed in two groups (group 1a and group 1b). The following stakeholders were represented:

- 13 academic research
- 2 chain stakeholders at farm level and above (breeders, feed industry, vets, ...)
- 3 chain stakeholders below the farm level (slaughterhouse, processing industry, retailers, consumers...)
- 1 engineer

Instrumental methods: 17 participants discussed about sensory methods with potential application in the pig industry. The following stakeholders were represented:

- 9 academic research
- 2 chain stakeholders at farm level and above (breeders, feed industry, vets, ...)
- 4 chain stakeholders below the farm level (slaughterhouse, processing industry, retailers, consumers...)  
- 1 engineer
- 1 administration

Further, as part of the workshop program, a video demonstration of the human nose methodology in carcasses at the slaughterline and a practical demonstration of the human nose methodology applied on boar fat samples was conducted.
Conclusions specific for sensory methods
Sensory methods were considered as useful, at least in the short term, but possibly also in the long term if it happens that no reliable instrumental method can be developed in a foreseeable future.

There is however a lot of concerns about the reliability of the human nose methods as they are currently applied by the industry (one line assessment by one or two people at chain speed). Those concerns are based on general considerations about olfactory noise, lack of time to perform the test, fatigue of the assessor, possible carry over effects from heavily tainted carcasses. The actual performance of the human nose methods is however very poorly documented and there is no strong evidence that they perform badly, nor that they perform in a satisfactory way. The gold standard to be used to assess the accuracy of the method is a very critical point here (see below).

During the workshop some of the cost assessments related to the human nose methodology were challenged by one of the major slaughterhouses in Europe, by not representing today’s industrial reality the way the on-line method is currently practised at the slaughterline.

Conclusions common for both sensory and instrumental methods
There is no need for harmonisation of the methods used to detect boar taint. Only the result (good accuracy to detect boar taint) is important, whereas the method to achieve that accuracy is not. Reference is made to the carcass classification scheme in which any method can be used provided that it has been approved on the basis of its accuracy.

There is however a need for harmonisation of the way in which the performance and accuracy of the methods is measured.

Yes / No (accepted / rejected) scaling is generally considered as the only realistic way of managing financial transactions between farmers and slaughterhouses. Multipoint scaling might however be useful for slaughterhouses to manage the use of tainted meat according to the intensity of taint. It should be taken into account that the skatole and androstenone threshold levels differ between countries and therefore the actual values for “Yes/No” scaling might also differ. This means that the on-line boar taint detection system should have flexibility to be adjusted or set on the required skatole/androstenone level specific for the particular country.

Which “gold standard” to measure the accuracy of the detection methods?
It is universally recognized that the difference in consumer acceptance between boar meat and castrate or gilt meat is the very definition of boar taint. It is however also universally recognized that consumer tests cannot realistically be used as a reference to measure the accuracy of detection methods in daily routine.
There is therefore a need for an easier to measure criterion that is sufficiently closely related to the above-mentioned definition of boar taint to be considered as a realistic gold standard for boar taint.

A majority of participants considered that androstenone, skatole and indole (ASI) levels are the best available “gold standard”, mostly on the ground that they are instrumentally measured and therefore objective.

This view was however challenged by other participants on the ground that

- There is some evidence that the levels of these compounds do not explain the whole of boar taint.
- Some studies suggest that the evaluation of meat by an expert panel would be better related to consumer acceptance of boar meat than ASI levels.

Using sensory evaluation as gold standard is however very difficult:

- A vast majority of participants consider that a single expert, be (s)he extremely good at detecting boar taint, cannot be used as gold standard for boar taint.
- The use of expert panels as gold standard presents a lot of difficulties:
  - How and on what basis should it be trained, knowing that the use of expert panels is motivated by the assumption that ASI levels are not satisfactory?
  - Harmonisation between panels would be extremely difficult to achieve.

### 4.4 Comparison of prioritized methods (WP 4)

#### 4.4.1 Test and comparison of potential methods currently in development

**Test materials**

Test material was prepared by the DG Joint Research Centre, Institute for Reference Materials and Measurements (JRC/IRMM), Standards for Food Bioscience Unit (Geel, Belgium). The content levels of the samples were agreed with the project partners in the frame of a teleconference. These content levels were realised by spiking four lard samples with portions of the analytes. For this purpose, the lard was melted at 40 °C and after spiking stirred over night with a magnetic bar. The lard was pipetted into screw cap vials and thereafter stored at – 20 °C. An overview of prepared samples is given in Table 11.

The test materials were analysed with an analysis method that was developed at the Joint Research Centre (JRC) and validated by collaborative trial. The analysis method is based on separating the analytes from the fat matrix by size exclusion chromatography followed by isotope dilution liquid chromatography tandem mass spectrometry.

**Table 11.** Analyte contents of the prepared test samples.
The homogeneity of the material was assessed by taking randomly 10 units and analyse them in duplicate. The results were analysed according to ISO-13528 [i] and IUPAC guidelines [ii], all samples were found to be homogenous. As an example, results of homogeneity testing of sample 3 are displayed in Table 12.

<table>
<thead>
<tr>
<th>Sample 1 lard</th>
<th>Indole [ng/g]</th>
<th>Skatole [ng/g]</th>
<th>Androsteneone [ng/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>105</td>
<td>104</td>
<td>4114</td>
</tr>
<tr>
<td>Sample 2 lard</td>
<td>386</td>
<td>1150</td>
<td>1159</td>
</tr>
<tr>
<td>Sample 3 lard</td>
<td>1145</td>
<td>362</td>
<td>336</td>
</tr>
<tr>
<td>Sample 4 lard</td>
<td>402</td>
<td>354</td>
<td>278</td>
</tr>
</tbody>
</table>

The test materials were shipped to the participating laboratories on dry ice, by express courier. Delivery of the samples occurred within 48 hours after dispatch.

The participants in the method comparison study received besides spiked lard samples also a portion of the refined lard sample, which was used for the preparation of the test materials. The participants were instructed to melt the lard at 40°C and homogenize it prior to analysis.

The stability of the samples was verified employing an isochronous scheme where three units of each sample were stored at -70 °C at the beginning of the study. After about 15 weeks the three units stored at -70 °C and three units stored at the recommended storage temperature (-20 °C) were analysed under repeatability conditions. The two sets of results were compared for significant differences. All samples but one were found to be stable over the period of the study. A slight decrease of the content of indole (~-15%) was found in sample 3, which represents the

---

**Table 12.** Individual results of homogeneity testing for sample 3.

<table>
<thead>
<tr>
<th>Bottle</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1085</td>
<td>1150</td>
<td>382</td>
<td>343</td>
<td>383</td>
<td>340</td>
</tr>
<tr>
<td>2</td>
<td>1103</td>
<td>1133</td>
<td>355</td>
<td>442</td>
<td>312</td>
<td>309</td>
</tr>
<tr>
<td>3</td>
<td>1166</td>
<td>1202</td>
<td>329</td>
<td>336</td>
<td>361</td>
<td>341</td>
</tr>
<tr>
<td>4</td>
<td>1288</td>
<td>1202</td>
<td>358</td>
<td>368</td>
<td>309</td>
<td>329</td>
</tr>
<tr>
<td>5</td>
<td>1066</td>
<td>1104</td>
<td>376</td>
<td>341</td>
<td>343</td>
<td>318</td>
</tr>
<tr>
<td>6</td>
<td>1057</td>
<td>1123</td>
<td>395</td>
<td>364</td>
<td>331</td>
<td>327</td>
</tr>
<tr>
<td>7</td>
<td>1168</td>
<td>1152</td>
<td>344</td>
<td>348</td>
<td>352</td>
<td>320</td>
</tr>
<tr>
<td>8</td>
<td>1148</td>
<td>1152</td>
<td>362</td>
<td>350</td>
<td>350</td>
<td>332</td>
</tr>
<tr>
<td>9</td>
<td>1077</td>
<td>1120</td>
<td>376</td>
<td>350</td>
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<td>339</td>
</tr>
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<td>10</td>
<td>1178</td>
<td>1234</td>
<td>363</td>
<td>359</td>
<td>346</td>
<td>328</td>
</tr>
</tbody>
</table>
highest content level. However, the effect on the study should be marginal as the period of the study was much shorter.

**Instrumental methods**

The most relevant prioritized instrumental methods based on the conclusions from the workshop (WP3), i.e. MS-based techniques (SIFT-MS, RF-MS, and PTR-MS), insect-based biosensors and electrochemical sensors were suggested for further method comparison tests. However, due to lack of time and labor of the invited participants, the RF-MS, PTR-MS and electrochemical sensor based methods could not be included in our method comparison study. Therefore, only two of the selected methods could be further tested; The SIFT-MS and the insect based biosensor methodology.

**SIFT-Mass spectrometry**

SIFT-MS uses soft chemical ionization reactions coupled with mass spectrometric detection to rapidly quantify Volatile organic compounds (VOCs) in real time from whole-gas samples (Figure 7). Three standard chemical ionization agents (or reagent ions) are used in SIFT-MS: H$_3$O$^+$, NO$^+$ and O$_2$$^+$. These reagent ions are mass selected and react with trace VOCs in very well controlled ion-molecule reactions but do not react with the major components of air, allowing SIFT-MS to analyse and quantify whole air for trace VOCs to parts per trillion on volume basis (pptv) levels. Mass selection of reagent ions means a switching time of 10 milliseconds, which enhances real-time selectivity compared to other technologies that use mechanical switching of different reagent source gas streams.

![Figure 7](https://example.com/schematic.png)

*Figure 7. Schematic representation of the SIFT-MS technique. Copyright Syft Technologies Ltd (2013).*

Soft chemical ionization yields a smaller number of product ions per compound than electron impact mass spectrometry (as used in GC/MS, for example), so gas chromatographic separation is unnecessary. This speeds sample throughput and provides instantaneous quantification of VOCs. Use of multiple reagent ions also greatly reduces interferences, markedly increasing the selectivity of SIFT-MS compared with most other whole-gas analysis technologies.
Released in 2014, the Syft Voice200ultra offers the latest developments in commercialised SIFT-MS instrumentation (Figure 8). Two instruments were used in this study:

- A Voice200ultra with high-performance inlet for direct air analysis. Here, the high-performance inlet was operated at 120 °C (it is designed for operation up to 200 °C). Transfer of the headspace sample to the instrument from an incubated sample bottle was by means of a passivated sampling needle. Heat transfer to the needle occurs from the high-performance inlet and is assisted by heating the needle prior to analysis with a heat gun.
- A Voice200 with a swab desorber inlet for analysing residues from surfaces. Here, the inlet was heated to 200 °C and used for characterising androstenone for detection using SIFT-MS.

These inlets are shown in Figure 8 and their use will be noted at appropriate points in the following discussion.

**Figure 8.** (a) Photograph of the Syft Technologies’ Voice200ultra instrument and engineering drawings of the two inlets used in this work: (b) the high performance inlet and (c) the swab desorber. Copyright Syft Technologies Ltd (2013).

Four experiments were conducted:

- **Experiment 1:** Detection of target compounds.
- **Experiment 2:** Background observed for rendered pig fat.
- **Experiment 3:** In-house spiked lard.
- **Experiment 4:** Blind spiked lard test samples
Experiment 1: Detection of target compounds

The first experiment had the purpose to confirm detection of target compounds using SIFT-MS based on commercially available substances, although the chemicals were approximately four years old.

SIFT-MS full scan mass spectra were obtained under the following conditions:

- Indole and skatole: a few crystals were placed in a 500 mL Schott bottle, capped with a pierceable septum and incubated at 50 °C for approximately one hour.
- Androstenone: one small crystal was dissolved in dichloromethane and a small fraction applied to a Teflon fabric swab. The dichloromethane was evaporated and the swab subsequently analysed via desorption at 200 °C using a Syft Voice200 instrument equipped with a Syft swab desorber inlet.

Full mass scans of indole, skatole and androstenone with each SIFT-MS reagent ion are shown in Figure 9. Indole and skatole products agree with work published previously (Wang et al., 2004), whereas androstenone has not previously been published. We note that the androstenone spectra also contain peaks that arise from impurity compounds.

Figure 9. SIFT-MS full scan mass spectra of (a) indole, (b) skatole, and (c) androstenone. Reaction mechanisms corresponding to observed peaks are shown.

a. Indole

\[
\text{Indole} + \text{H}_3\text{O}^+ \to \text{Indole.H}^+ (m/z 118) + \text{H}_2\text{O} \quad \text{Proton transfer}
\]

\[
\text{Indole} + \text{NO}^+ \to \text{Indole}^+ (m/z 117) + \text{NO} \quad \text{Electron transfer}
\]

\[
\text{Indole} + \text{O}_2^+ \to \text{Indole}^+ (m/z 117) + \text{O}_2 \quad \text{Electron transfer}
\]
b. Skatole  
(131 amu)

N.B. the sample appears to contain significant impurities; product ions are marked with an asterisk.

These results demonstrate that all three compounds are readily detected using each of the three reagent ions in SIFT-MS. By using three rapidly switchable reagent ions, SIFT-MS offers enhanced selectivity over techniques employing a single reagent ion or harsher ionisation methods coupled with quadrupole mass spectrometric detection.

Experiment 2: Background observed for rendered pig fat (lard)

Earlier work suggests that the headspace of lard is chemically complex even at room temperature. This experiment was performed to evaluate the complexity observed in a quadrupole-based SIFT-MS instrument.

Rendered pig fat (lard) was sourced from a local butchery in Christchurch, New Zealand.

Lard (50 grams) was incubated at 70 °C for one hour in a 500 mL Schott bottle and capped with a pierceable septum.

The SIFT-MS full mass spectra of lard at 70 °C are shown in Figure 10. They reveal a complex mixture of volatile organic compounds is present at 70 °C and concentrations...
of these are significant in both the indole (m/z 117 or 118) and skatole (m/z 131 or 132) regions of the spectrum. Note that a signal of 1000 Hertz (counts per second) corresponds very approximately to a concentration of 10 ppbv in the headspace.

The background VOC levels of commercially sourced lard are significant even at 70 °C, confirming the work done by Schafer et al. using PTR-TOF-MS.

Given the relatively low volatility of androstenone, it is likely that a practical slaughterhouse implementation will require heating of samples to approximately 200 °C (c.f. the work of Schäfer et al. 2011). At this temperature, higher levels of volatiles will be evaporated from the fat and chemical complexity will increase as temperature-induced reactions occur rapidly (i.e. cooking). These factors suggest to us that the quadrupole mass spectrometric detection currently used in commercial SIFT-MS instruments may not provide sufficient selectivity for testing taint compounds in boar fat.

Figure 10. SIFT-MS full scan mass spectra (H$_3$O$^+$, NO$^+$ and O$_2$$^+$ reagent ions) of lard at 70 °C.

Experiment 3: In-house spiked lard

Preliminary assessment of detection capability of a Syft Voice200ultra SIFT-MS instrument at specified concentrations with available sample introduction approach (high-performance inlet on Voice200ultra; samples heated to 70 °C).

Lard was spiked with skatole and androstenone at concentrations of 5 mg / kg (similar to the upper concentration indicated by Thomas Wenzl (JRC/IRMM) for the comparative test samples). Dichloromethane (DCM) was used as the solvent for skatole and androstenone because it reacts very slowly with the H$_3$O$^+$ and NO$^+$ reagent.
ions of SIFT-MS, so any residual solvent has minimal effect on analysis carried out using these ions.

Lard samples (50 grams) were melted, the spike added (50 µL), the sample was capped with a pierceable septum, and thorough mixing undertaken. The sample was then incubated at 70 °C for about one hour prior to analysis.

**Table 13** summarises the results obtained for lard samples spiked with skatole and androstenone at the upper limit (5 mg / kg).

**Table 13.** Concentrations of skatole and androstenone in lard, lard with a blank spike of DCM, and lard with skatole and androstenone spiked in DCM, using SIFT-MS with incubation at 70 °C.

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Lard / ppbv</th>
<th>Lard with DCM spike / ppbv</th>
<th>Lard with target compounds / ppbv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skatole</td>
<td>84</td>
<td>130</td>
<td>181</td>
</tr>
<tr>
<td>Androstenone</td>
<td>4.5</td>
<td>5.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Skatole is readily detected at 70 °C when spiked at the upper end of the expected concentration range in boar fat. Androstenone is barely detected, confirming the approach taken by Schafer et al. in heating the samples to >160 °C. Because androstenone detection was poor at 70 °C even at the highest spiking level, experiments at lower spike concentrations were not conducted.

**Experiment 4: Blind spiked lard samples from Belgium**

Because of the marginal results obtained in Experiment 3, it was decided by the participant not to proceed on testing the blind spiked samples of the method comparison study, because it was unlikely to expect a positive outcome.

**Insect based biosensors**

This measurement principle is based on insects (parasitic wasps) ability to learn and respond to particular concentrations odour compounds. In particular, it has been demonstrated that they are able to learn and discriminate the boar taint compounds, skatole, androstenone and indole (Olson et al 2012). The biosensor based method relies on an initial calibration step that requires that the insects (parasitic wasps) are trained to known concentrations of pure single boar taint substances skatole and androstenone in boar fat as single compounds.

Only one sample with all the three boar taint substances indole, skatole and androstenone at known concentrations was provided together with the tests samples for the method comparison study from calibration to be used for the learning of the wasps.

As initial responses to the test samples were weak after the learning phase, it was attempted to habituate the wasps to the boar fat by placing 3 petri dishes with pure
(unspiked) boar fat at the bottom of a cage with the trained wasps for 15-20 min. prior to the testing.

None of the 42 wasps trained and tested to the boar fat sample responded. In addition, none of the wasps tested to the pure androstenone after evaporation of the DCM showed any response. The androstenone used in this study has a different lot number than in our previous studies. A technical support scientist at Sigma-Aldrich stated that the compound was 98.5% pure based on thin layer chromatography analysis, but that some un-reported contamination in the lot could be present that was not in our original batch. Regardless of the reason for the results found here, this could have overestimated the concentrations in the boar fat samples.

It was then realized that the received calibration was not suitable for the insect learning/training procedure.

The training of the wasps to known concentrations of skatole and androstenone in boar fat as single compounds would be a more effective means of training the wasps for these tests.

Since such calibration samples were not available at the time of testing, the performance of this method could not be properly evaluated.

### 4.4.2 Comparison of methods currently in use at industry level

The human nose methodology is currently used in several slaughterhouses in Europe to detect boar taint online. Data regarding the performance of this methodology is currently lacking. Aim of this task was therefore to evaluate the performance of the online methods currently in use in several European countries.

One aim of the DG SANCO project is to evaluate the performance of the online boar taint detection methods, currently in use at industry level. Requirements regarding this method are speed, sensitivity, reproducibility and accuracy (Table 14).

<table>
<thead>
<tr>
<th>Detection methods</th>
<th>For boar taint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity/ Analysis speed</td>
<td>High number of samples in short time</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Enough to be able to identify low levels of boar taint</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Sufficient</td>
</tr>
<tr>
<td>Accuracy – correct classification rate</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Sensitivity is a measure for the proportion of actual positives which are correctly identified (= number of true positives / [number of true positives + number of false negatives]).
Specificity indicates the proportion of negatives which are correctly identified as such (= number of true negatives / [number of true negatives + number of false positives]). These criteria can be used to evaluate the performance and reliability of the methods in use, but require a golden standard for boar taint.

This can be chemical analysis of the boar taint compounds or sensory evaluation by an expert panel or an expert online. Up to now, there are no certified experts, evaluating boar taint online. The boar taint compounds – androstenone, skatole, indole – can be used as an objective reference, but chemical analysis of these compounds in fat is expensive (at least 70 € per sample). Expert panel evaluation is another possibility. For these expert panels it is valuable that panel performance results compared to chemical analysis as well as inter and intra reliability are also reported.

According to the industrial investigations on boar taint detection methodology (WP1, deliverable report D1.2), boar taint detection is at least performed online in the following five countries (slaughter companies): The Netherlands (Vion), Belgium (Westvlees), Denmark (Danish Crowns), France (Cooperl) and Germany (Tönnies Fleisch). The slaughterhouses were contacted to question their willingness to participate in a trial to evaluate their evaluation system to detect boar taint online.

A study protocol was prepared (Annex 4) with an experimental design that aimed at evaluating at least 300 carcasses online by at least one trained expert assessor to investigate the performance of sensitivity and specificity according to Table 15.

**Table 15.** Human nose performance criteria.

<table>
<thead>
<tr>
<th>Condition (as determined by &quot;Gold standard&quot;)</th>
<th>Condition positive</th>
<th>Condition negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test outcome positive</strong></td>
<td>True positive</td>
<td>False positive</td>
</tr>
<tr>
<td><strong>Test outcome negative</strong></td>
<td>False negative</td>
<td>True negative</td>
</tr>
</tbody>
</table>

Precision = True positive / Σ Test outcome positive

Negative predictive value = True negative / Σ Test outcome negative

Accuracy =

Further, the following objectives were to be investigated:

- On-line sensitivity and specificity performance to be compared with the sensory evaluation of the fat samples by 3 sensory expert assessors at the Institute of Agriculture and Fishery Research (ILVO), Belgium.

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• The online method will be screened compared to chemical analysis and sensory evaluation of the University of Göttingen sensory expert assessors for all samples identified as having boar taint by the online method, 40 samples identified as without boar taint by the online method, all false negative samples (samples identified as without boar taint by the online method but identified as with boar taint by the ILVO experts) and all false positive samples (samples identified as with boar taint by the online method but identified as without boar taint by the ILVO experts).

• We intend to perform this evaluation in five major European slaughterhouses, one per country. In at least three slaughterhouses.

• Besides online evaluation, an extra at line evaluation will be performed to increase the number of slaughterhouse experts involved in the study.

In order to reduce the costs and increase feasibility, a second experimental set-up was also developed (Annex 4) to evaluate the at-line performance of the human nose experts, performing the online detection method. This protocol is not sufficient to evaluate the performance of the methods currently in use, but can already provide valuable information regarding the human nose methodology as a first prerequisite to have a good online evaluation is the selection and training of the experts as well as high intra and inter reliability. The evaluation of the experts at-line would have provided comparable results regarding inter and intra reliability at line, as well as olfactory acuity to perceive skatole and androstenone, of the experts in different European country. Unfortunately, an insufficient number of slaughterhouses was willing to participate.

The two on-line and at-line protocols were proposed to five slaughterhouses in Europe. The response to the on-line protocol was that three slaughterhouses indicated to agree with the experimental set-up of the protocol. One slaughterhouse raised questions regarding the reference method to evaluate the online performance. They suggested that it would be useful to have confrontation with human nose on line by expert on line. Besides, the 2 sensory lab experts of reference are different and they do not use the same methodology (microwave method versus hot iron method). They also indicated that sensitivity and specificity of the expert panels should be reported. Another slaughterhouse indicated that the protocol would not result in sufficient data on which to base decisive conclusions.

One slaughterhouse did indicate that it was willing to participate and pay the participation fee. However, the other four slaughterhouses were not able to participate due to time constraints, due to budget limitations, due to other reasons or a combination of the latter. Three slaughterhouses indicated that they were already involved in ongoing national projects.

The response to the at-line protocol was that several slaughterhouses indicated that they already performed tests to evaluate the at line human nose method for detecting boar taint. One company already published some data regarding at line performance(Mathur et al., 2012). Another company is using the “hot water method” routinely on a small number of boars to check at line performance. One slaughterhouse made a critical remark that the sensitivity and performance of the expert panels and their inter- and intra-reliability should also be reported.
Two slaughterhouses argued that the at-line method is not an issue when performing a limited number of assays. Research should focus on the on-line method that needs testing under full slaughter capacity conditions. However, they were also not willing to participate in the online test, which made it impossible to do so.

To realize the industrial study, it was assumed that the slaughterhouses covered their own labor and sample costs. However it turned out, that the study could not be conducted due to limited resources among the slaughterhouses. Slaughterhouses were not willing to participate in the method comparison study either due to time constraints, or they were not prepared or able to cover their labor and sample costs.
5. References

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Andresen, Ø. and H. Bakke 1975. 5α-androstenone in fat from boars selected for rate of gain and thickness of backfat, and from boars used in artificial insemination service. Acta Veterinaria Scandinavica, 16, 492-502.


Berdagué, J.L., Rabot, C., and M. Bonneau 1996. Rapid classification of backfat samples selected according to their androstenone content by pyrolysis-mass-spectrometry. Sciences des Alimentes. 16, 425-433.


Olson D.M., Rains G.C., Meiners T., Takasu T., Tertuliano M., Tumlinson J.H., Wäckers


6. List of abbreviations

amu  atomic mass unit
DCM  Dichloromethane
FTIR  Fourier Transform Infrared
GC   Gas Chromatography
HPLC  High Performance Liquid Chromatography
LC   Liquid Chromatography
LOQ  Limit Of Quantification
LR   Labour requirement
MS   Mass Spectrometry
PAS  Photo Acoustic Spectroscopy
PLC  Programmable Logical Control
ppm  parts per million, microgram per gram
ppb  parts per billion, nanogram per gram
pptv parts per trillion (picogram) on volume basis
PTR  Proton Transfer Reaction
PTR-MS  Proton Transfer Reaction Mass Spectrometry
RF-MS  Rapid Fire Mass Spectrometry
RF-MS  RapidFire mass Spectrometry
RFID  Radio-frequency identification
SIFT-MS  Selected Ion Flow Tube Mass Spectrometry
TOF  Time Of Flight
TOF-MS  Time Of Flight Mass Spectrometry
uPLC  ultra Performance Liquid Chromatography
VOC  Volatile Organic Vompounds
8. Annex 1: Questionnaire boar taint detection methods

Contact details
1) What is the name of your institution/company?
…………………………………………………………………………………………………………………………………………………………………………………………………………………………

2) What is your name?
…………………………………………………………………………………………………………………………………………………………………………………………………………………………

3) Your e-mail address in case of further inquiries?
…………………………………………………………………………………………………………………………………………………………………………………………………………………………

A) Measurement principle
Part A concerns specific questions about the measurement principle of your method

4) What type of method is it?
☐ Quantitative (measures absolute concentrations of levels of substances)
☐ Semi-quantitative (measures relative differences in levels of substances)
☐ Qualitative (yes or no/presence or no presence of boar taint substances or boar taint)

5) What is the measurement/detection principle of the method?
☐ Spectrophotometry
☐ Gas-phase fingerprinting/Gas-sensor array
☐ Mass-spectrometry
☐ Ion Molecule Reaction
☐ Immunology
☐ Biosensor
☐ Sensory perception with trained persons/Human nose
☐ Other(specify),…………………………………………………………………………………………………………………………………………………………………………………………………………………………

6) Which chemical compounds do you measure? (tick all that apply)
☐ Androstenone
☐ Skatole
☐ Indole
☐ Method is not compound specific, but is calibrated/indirectly correlated against a quantitative method that measures skatole, androstenone or both.
☐ Method is not compound specific but is calibrated/indirectly correlated against sensory perception of boar taint
☐ Other, ........................................................................................................................................................................

7) Does the method allow simultaneous or parallel detection of compounds?
☐ Yes
☐ No

8) Has the measurement principle for this application been published?
☐ Yes
☐ No
If Yes, please include the reference of the publication:................................................................................................
B) Sampling

Part B concerns general questions related to sampling for your method

9) Do you take a sample from the carcass to analyse elsewhere or do you analyse directly on the carcass/next to the carcass?
   - ☐ We take a sample from the carcass and analyse elsewhere
   - ☐ We analyse directly on the carcass
   - ☐ We take a sample from the carcass and analyse it at the point of test/next to the carcass

10) Where on the carcass do you take the sample or do you analyse directly on the carcass? (see picture for location of A, B, C)
    - ☐ A, the neck
    - ☐ B, the belly
    - ☐ C, the ham
    - ☐ We take the sample on another location as A, B or C, namely ......................

11) What is the composition of the sampling location on the carcass? (please tick all that apply)
    - ☐ It contains subcutaneous fat (e.g. neck fat)
    - ☐ It contains internal fat
    - ☐ It contains muscle tissue
    - ☐ It contains skin

12) What is the total amount of sample needed for the analysis? ............ gram

13) Does the method require any clean-up/extraction step before detection takes place?
    - ☐ Yes
    - ☐ No
    If Yes, please describe briefly the clean-up/extraction step
      ........................................................................................................................................................................
      ........................................................................................................................................................................
    If no, do you measure directly on the solid phase or the gas phase?
      ........................................................................................................................................................................

14) How do you treat the sample to detect boar taint (applies to sensory/human nose based method)?
    - ☐ We do not heat
    - ☐ Gas powered torch, which heats a contact plate, which is pressed to carcass/sample
    - ☐ Electric soldering iron, which is pressed to the carcass/sample
    - ☐ Microwave
    - ☐ Boiling in hot water
    - ☐ We heat but in another way as those above, namely by
      ........................................................................................................................................................................

15) Has the cleanup extraction procedure for this application been published
    - ☐ Yes
    - ☐ No
    If Yes, please include the reference of the publication: ......................................................................................

C) Validation state of the method

Part C relates to the extent of validation and verification of the method
16) Is the method still under development

☐ Yes
☐ No

If yes, please describe the current status of the method

…………………………………………………………………………………………………………………………………………………………

17) Is there a laboratory prototype measurement system/device available?

☐ Yes
☐ No

If yes,
☐ Only the detection part of the method exists as prototype
☐ The prototype system has an integrated sampling unit
☐ It is a complete integrated system including sampling and detection and with an operation and data analysis and reporting software

18) Is there a commercial prototype system available?

☐ Yes
☐ No

If yes,
☐ Only the detection part of the method exists as prototype
☐ The prototype system has an integrated sampling unit
☐ It is a complete integrated system including sampling and detection and with an operation and data analysis and reporting software

19) Has a feasibility study on real boar samples been carried out?

☐ Yes
☐ No

If Yes,
Please specify the type and number of samples analysed in the study.

Has the study been published yet?

☐ Yes
☐ No

If yes, please include the reference of the publication (and if feasible, please submit an electronic version of the published paper(s) together with the submission of the filled in questionnaire:

…………………………………………………………………………………………………………………………………………………………

20) Has the method been externally validated in a collaborative study or inter-laboratory comparison study?

☐ Yes
☐ No

If yes:

21) Has the study been published?

☐ Yes
☐ No
If yes, please include the complete reference of the publication (and if feasible, please submit an electronic version of the published paper(s) together with the submission of the filled in questionnaire):

…………………………………………………………………………………………………………………………………………………………

22) Are you currently applying the method for routine analysis of boar samples?

☐ Yes
☐ No

D) Method performance characteristics

Part D relates to the performance of the method according to ISO-3534-2 standard

23) What is the concentration range of boar taint substances where the method is applicable? ..........nanogram/microgram/gram (fat or liquid)

24) What is the repeatability of the method (identical sample measured, same measurement procedure, same operator, same conditions)? .................................%

25) What is the accuracy/trueness of the method (closeness of agreement between a test or measurement result and the true/reference value)?.........................%

26) What is the limit of detection (3 times standard deviation of blank value signal of a real sample, i.e., including the matrix background)............... nanogram микро грам/gram (fat or liquid)

27) Could you indicate the following method characteristics (applies to sensory/human nose based method)

☐ the sensitivity (percentage correctly classified tainted samples) is .........................%  
☐ the specificity (percentage of correctly classified untainted samples) is ........................%  

E) Analysis speed

Part E relates to the time needed for carrying out the method/analysis

28) Please indicate the estimated time needed for (thick and specify only what applies)

☐ Sampling..........................seconds/minutes
☐ Extraction....................... seconds/minutes
☐ Total cleanup time including extraction.............seconds/minutes
☐ Measurement/Detection...............seconds/minutes
☐ Data analysis/result treatment.............seconds/minutes
☐ Total analysis time..................seconds/minutes
☐ Time between consecutive measurements

29) What is the estimated sample throughput/capacity of the method (including replicates, controls/calibrants).........samples/hr
F) Automation potential for use at slaughterline

Part F relates to the automation potential for future use at the slaughter line

30) Can the method be adapted to the slaughter line?

☐ Yes
☐ No

31) Can the sampling be done directly on the carcass in the slaughter line

☐ Yes
☐ Yes

32) Can the sampling procedure be automated?

☐ Yes
☐ No

33) Can the measurement/detection principle be automated?

☐ Yes
☐ No

34) Can the data analysis and result reporting be automated?

☐ Yes
☐ No

35) Will highly qualified staff be needed for operating the method at the slaughter-line?

☐ Yes
☐ No

36) Can the measurement system be easily technically maintained?

☐ Yes
☐ No

37) Can this technology be incorporated/linked with other measuring systems used in abattoirs?

☐ Yes
☐ No

38) Can the analysis result be available immediately after the measurement?

☐ Yes
☐ No

39) Can this technology be incorporated/linked with other measuring systems used in abattoirs?

☐ Yes
☐ No

G) Estimated costs of the method

Part G concerns questions about the costs of your boar taint detection method.

In case of a not yet fully developed method fit for purpose:
40) Estimated development costs still needed for establishing a fully in-house validated method? (in local currency):.................................

41) Estimated person months needed for establishing a fully in-house validated method? ........months/years

42) Estimated time needed for establishing a fully in-house validated method? ........months/years

43) Estimated hardware development costs for fully developed measurements system/device prototype (in local currency):.................................

In case of a fully developed and validated method:

44) Estimated investment /implementation cost for the industry/slaughterhouse company of the measurement system/device (in local currency)?.................................

45) Estimated total annual running costs of the measurement system/device (in local currency)..................

H) IPR issues

46) Are there any IPR issues related to your method?
   □ Yes
   □ No

   If No:
   47) Can your data be used in the deliverable report that will come out of the WP1 of the BoarCheck project?

   If Yes:
   48) What are the restrictions with regard to including your data in our BoarCheck project WP1 reporting?

49) Has the method/measurement principle or parts of the method been patented?
   □ Yes
   □ No

50) Has the method/measurement principle or parts of the method been filed?
   □ Yes
   □ No

51) Could you provide us with the patent or file number?
   □ Yes
   □ No
   If yes, we would appreciate if please could include a copy of the respective document with the return of the filled in questionnaire?

You have completed the questionnaire. Thank you very much for your participation. Please return the questionnaire via email to the contact person in the accompanying letter.

Contact details

1) What is the name of your company? ..............................................................................................................

2) What is your name? ...........................................................................................................................................

3) What is your function within your organization? ...........................................................................................

General questions

4) What is the average number of pigs (female pigs, barrows, entire male pigs and immunocastrates together) your company currently slaughters per week? ......................................................... pigs per week

5) What is the average slaughter line speed? ......................... pigs slaughtered per hour

6) Do you currently slaughter entire male pigs?
   □ No, and we are not planning to do this in the next years
   □ No, but we do expect to start to slaughter entire male pigs in the next years
   □ Yes

7) If you do slaughter entire male pigs, what is the average number of entire male pigs your company currently slaughters per week? ..................................

8) Do you currently slaughter immunocastrated male pigs?
   □ No, and we are not planning to do this in the next years
   □ No, but we do expect to start to slaughter immunocastrated male pigs in the next years
   □ Yes

9) If you do slaughter immunocastrated male pigs, what is the average number of immunocastrated male pigs your company currently slaughters per week? ..............

10) Do you currently have a system in place for the detection of boar taint?
    □ No
    □ Yes

If you have a system in place for the detection of boar taint, please complete part A, B, C and D of the survey. If you do not have a boar taint detection system yet, please only complete part A of the survey.

A) Requirements for a boar taint detection system at the slaughter line

Part A of the survey concerns requirements for an accurate and rapid boar taint detection system at the slaughter line. What requirements to such system are minimal in your point of view?

11) Do you think boar taint detection at the slaughter line is necessary?
    □ No
    □ Yes

12) If you think a boar taint detection system is necessary, what would be the maximum costs your company is prepared to pay for it (in local currency)? ......................................................... per tested entire male pig

13) What kind of measurement scale of boar taint is necessary?
    □ A scale with only ‘boar taint’ and ‘no boar taint’ (two point scale)
    □ A scale with more than two levels measuring boar taint, namely with ........................................ levels

14) Where would you locate a detection system in the slaughter line?
    □ Directly after killing and before bleeding
    □ Directly after bleeding and before the meat inspection
    □ Directly after the meat inspection
    □ In the cooling area
    □ Elsewhere, namely ........................................................................................................................................

15) How important do you consider each of the following aspects for a boar taint detection system? (1 = not important, 5 = very important)
16) Is it relevant for you as a slaughter company to report the results of the boar taint detection to the pig producer?
☐ No
☐ Yes

If you do not have a boar taint detection system, you are finished and do not need to fill out the remainder of the survey. Thank you very much for your participation.
If you have a boar taint detection in place in your slaughter plant(s), please go to part B of the survey.

B) Characteristics of the system in use in your slaughter company
Please first complete part B1 of the survey, which concerns general questions valid for analytical and sensory boar taint detection systems.

B1) General characteristics of the system in use in your slaughter company

17) Which method do you use for detecting boar taint at the slaughter line?
☐ Sensory analysis with the human nose
☐ Chemical analysis

18) Are the analyses for boar taint routine (all entire male pigs) or selective (a sample)?
☐ Routine, all entire male pigs slaughtered
☐ Selective, a sample of all entire male pigs slaughtered

19) In our boar taint detection system we define boar taint based on ... (tick all that apply)
☐ Androstenone concentration via chemical analysis
☐ Skatole concentration via chemical analysis
☐ Androstenone smell via sensory evaluation by trained humans
☐ Skatole smell via sensory evaluation by trained humans
☐ Deviant smell or off-odour by trained humans
☐ Another aspect than above, namely .................................................................

20) What type of scale do you use to measure boar taint?
☐ A scale with only ‘boar taint’ and ‘no boar taint’ (two point scale)
☐ A scale with more than two levels measuring boar taint, namely with .................................... levels

21) What is the average percentage of carcasses in each level of boar taint distinguished in question 18?
Level 1: ......................... %
Level 2: ......................... %
Level 3: ......................... %
Level 4: ......................... %
Level 5: ......................... %
Level 6: ......................... %

22) At what average slaughter line speed do you test for boar taint? ...................... entire male pigs per hour
23) What is the average analysis time per carcass? ................................................ seconds/carcass
24) Where at the slaughter line is your boar taint detection system located?
☐ Directly after killing and before bleeding
☐ Directly after bleeding and before the meat inspection
☐ Directly after the meat inspection
☐ In the cooling area
☐ Elsewhere, namely .................................................................
25) Do you take a sample of the carcass to analyse elsewhere or do you analyse directly on the carcass?
☐ We take a sample from the carcass and analyse elsewhere
☐ We analyse directly on the carcass
26) Where on the carcass do you take the sample or do you analyse directly on the carcass? (see picture for location of A, B, C)
☐ A, the neck
☐ B, the belly
☐ C, the ham
☐ We take the sample on another location as A, B or C, namely ....................
27) What is the composition of the sample or location on the carcass? (please tick all that apply)
☐ It contains subcutaneous fat (e.g. neck fat)
☐ It contains internal fat
☐ It contains muscle tissue
☐ It contains skin
28) Do you measure the efficiency and accuracy of your boar taint detection system?
☐ No
☐ Yes. Could you indicate the following testing quality aspects?
☐ the sensitivity of my system (percentage of false negatives) is ......................% (% of carcasses with boar taint are classified as without boar taint)
☐ the specificity of my system (percentage of false positives) is .....................% (% of carcasses without boar taint are classified as with boar taint)
☐ the reproducibility of my system is .......... % (degree of agreement between paired measurements of different assessors/devices on same entire male pig)
☐ the repeatability of my system is ...........% (degree of agreements between paired measurements of same assessor/device on same entire male pig)
29) Do you report the results of the boar taint assessment back to the pig producer?
☐ No
☐ Yes

If your boar taint detection system uses sensory evaluation (human nose), please go to part B2 of the survey. If it uses chemical analysis of specific compounds, please skip part B2 and go to part B3 (on p6).

B2: Sensory method (human nose)
Part B2 concerns specific questions about your boar taint detection system based on a sensory method.

30) What is the maximum time an assessor is allowed to assess carcasses continuously? ....................... minutes
31) What is the minimum break between two periods of assessment? ................................................................. minutes
32) What is the interval between evaluating two consecutive carcasses by the same assessor? ................. seconds
33) How many samples/carcasses does a single assessor evaluate on an average day? ......................... carcasses
34) How many assessors do you use to assess one carcass?
☐ one
☐ More than one, namely ...................................................................................................................
35) How do you heat the carcass/sample to detect boar taint?
☐ We do not heat
☐ Gas powered torch, which heats a contact plate, which is pressed to carcass/sample
☐ Electric soldering iron, which is pressed to the carcass/sample
☐ Microwave
☐ Boiling in hot water
☐ We heat but in another way as those above, namely by ........................................

36) How do you select and train assessors? (Please tick all items which are applicable)
☐ We don’t have a selection and training procedure
☐ Assessors should be sensitive to androstenone
☐ Assessors should be sensitive to skatole
☐ Assessors should show satisfactory performance in a laboratory setting
☐ Assessors should show satisfactory performance in a slaughter line setting

37) If you have one, how long is the selection and training period? ...................... weeks

38) If you have one, how much training does an assessor receive? ....................... hours

39) How often do you evaluate assessor performance?
☐ Not
☐ Daily
☐ Weekly
☐ ........................................ times per month
☐ ........................................ times per year

40) If assessor performance is evaluated, this is done by
☐ chemical compounds (e.g. androstenone, skatole)
☐ cross validation with other assessors
☐ another method, namely ...........................................................

You have completed the specific questions concerning your boar taint detection system based on sensory evaluation. Please go to part C (on p6) of the survey concerning the associated costs.

B3: Analytical method
Part B3 concerns specific questions about your boar taint detection system based on chemical analysis of specific compounds.

41) Which type of analytical method do you use?
☐ Spectrophotometry
☐ Gas-phase fingerprinting mass-spectrometry
☐ Ion Molecule Reaction
☐ other, ............................................................................................................................

42) Which chemical compounds do you measure? (tick all that apply)
☐ Androstenone
☐ Skatole
☐ Other, ...........................................................

43) How often do you assess the performance of the device to detect boar taint?
☐ Not
☐ Daily
☐ Weekly
☐ ........................................ times per month
☐ ........................................ times per year

You have completed the specific questions concerning your boar taint detection system based on chemical analysis of specific compounds. Please go to part C of the survey concerning the associated costs.

C) Costs of the system in use
Part C concerns questions about the costs of your boar taint detection system. The questions are the same for detection systems based on sensory evaluation and on chemical analysis.

44) Total initial investment costs of the system (in local currency) were ………………………
45) Total running costs of my boar taint detection system (in local currency) are ……………………….. per slaughtered entire male pig

You have completed the questions concerning the costs of your boar taint detection system. Please go to part D of the survey.

D) Use of meat from carcasses or cutting parts with boar taint

Part D concerns questions about the use of meat or cutting parts from carcasses assessed as with boar taint. The questions are the same for detection system based on sensory evaluation and on chemical analysis.

46) Do you receive boar taint related complaints from customers / retailers/ consumers?
   ☐ No
   ☐ Yes. We receive on average …………………………………………… complaints per month
47) What do you do with meat from entire male pigs assessed as having boar taint? (tick all that apply)
   ☐ We sell this meat on a specific market, that accepts meat from entire male pigs with boar taint
   ☐ We use this meat in dried products that are expected to be consumed without heating
   ☐ We use this meat in cured products that are expected to be consumed without heating
   ☐ We use this meat in cooked products that are expected to be consumed without heating
   ☐ We use seasoning to mask the boar taint
   ☐ We mix it with non-tainted meat to such extent that it does not lead to complaints.
   ☐ We use this meat in products that have undergone another process, namely ……………………………………………………………………………………………………………………………
48) Do you have clients that refuse meat from entire male pigs
   ☐ No
   ☐ Yes, namely ……………………………………………………………………………………………………………………………

You have completed the questionnaire. Thank you very much for your participation. Please return the questionnaire via email or regular mail to the contact person in the accompanying letter.
10. Annex 3: Instructions method comparison study

BoarCheck - Method comparison of skatole and androstenone analyses
(based on Thompson et al., 2006)

Background

As part of the ongoing DG-SANCO EU project BoarCheck, (SANCO/2012/11505 - A study on rapid methods for boar taint used or being developed at slaughter plants in the European Union), that aims at identifying rapid boar taint detection methods with potential for future use at slaughter plants, one of the tasks is to carry out a method performance comparison study on the methods which have so far been identified to have the most promising potential for future rapid detection on the slaughterline.

The partners involved in this project are Nofima AS (Norway), Instituut voor Landbouw- en Visserijonderzoek ILVO (Belgium), Institut de Recerca i Tecnologia Agroalimentaries IRTA (Spain), Danish Meat Research Institute DMRI (Denmark), Institut de la Filière Porcine IFIP(France), LEI Wageningen UR (Netherlands), Georg-August-Universität Göttingen (Germany) and University of western England (United Kingdom).

The selection of your method has been based on its ability to fulfil the industrial method performance requirements for rapid boar taint detection on the slaughterline. The aim of this study is to obtain further documentation on the methods performance with regard to accuracy and repeatability. Among the selected methods which will be further evaluated are both instrumental, biosensor and sensory based methods.

We would highly appreciate your participation in this study. This study is an excellent opportunity to further evaluate your methodology against other potential industrial methods. The results of the study will be used to provide a list of the currently most promising rapid methods to detect boar taint on carcasses from entire and immunocastrated male pigs to be used in slaughter plants in future in the EU.

Rationale for the proficiency testing

For an instrumental method to produce consistently reliable data, it must implement an appropriate program of quality-assurance and performance-monitoring procedures. Proficiency testing is one of these procedures. The usual format for proficiency testing schemes is based on the distribution of samples of a test material to the participants. The participants analyze the material without knowledge of the correct result and return the result of the measurement to the scheme provider. The provider converts the results into scores that reflect the performance of the participant laboratory. This alerts the participant to unexpected problems that might be present, and spurs the management to take whatever remedial action is necessary. The ethos of the Protocol (Thompson et al., 2006) is that proficiency testing should provide information on the fitness-for-purpose of analytical results provided by participants, to assist them in meeting requirements.

Thompson’s Protocol is applicable where the principal aim is the assessment of laboratory performance against the established assigned values.

Framework of BoarCheck proficiency test
Test materials will be distributed to the participants, who are required to return results by a given date. A value is assigned for each measurand which is not disclosed to participants until after the reporting deadline. The results are subjected to statistical analysis by the scheme provider, and participants are notified of their performance. Advice will be available to poor performers, and all participants will be kept fully informed of the progress of the scheme.

Proficiency test scheme provider

The proficiency testing scheme provider responsible for the organisation and coordination of the proficiency test is Nofima Mat AS, Norway.

Test material

The test material consists of pure fat originating from boar backfat tissue. Samples, each of 10 g at 3 levels, of purified fat have been formulated with respectively indole, skatole and androstenone. The test material has been checked for homogeneity.

Analytes to be measured

The laboratory should analyse and quantify skatole (3-methylindole) and androstenone (5α-androst-16-en-3-one). Optionally, it is encouraged also to analyse and report indole, if the method also measures this compound.

Analysis strategy

Basically only one analysis/determination should be performed on each sample, and the respective results reported. This means that no replicates should be analysed and mean results reported. Reporting the mean of replicate determinations on proficiency test samples should be carried out only if this is the norm for routine work. (Procedures used by laboratories participating in proficiency testing schemes should simulate those used in routine sample analysis.)

Note: Separate reporting of results replicated within laboratories is allowed as a possibility in proficiency tests, but is not recommended. If the practice is followed, scheme providers and participants must beware of misinterpreting repeatability standard deviations averaged over many participants. For example, the within-group sum of squares obtained by analysis of variance cannot be interpreted as an “average” repeatability variance when different analytical methods are in use.

Reporting of results by participants

Participants must report results in the format required according to the Excel sheet distributed to each laboratory: Concentrations will be reported in μg/g fat (ppm) with 3 decimals. Results lower than LOD should be reported as less than a number. Deadline for reporting is 25th May 2014. Submitted results cannot be corrected or withdrawn. Results submitted after the deadline must be rejected.

Note: The reason for this strict approach is that proficiency testing is meant to test every aspect of obtaining and producing an analytical result, including calculating, checking, and reporting a result (Thompson et al. 2006).

The completed Excel file should be returned electronically to the test provider:
The participating laboratory should include a brief outline of the analysis protocol(s) being used comprising both sampling/extraction, analysis, detection, quantification, limit of detection (LOD) and how LOD is defined. In case the participant reports mean values based on replicate measurements, this should be stated.

Assessment of performance
Labs will be assessed on the difference between their result and the assigned value. A performance score will be calculated for each laboratory, using the statistical scheme detailed in Section 3.1., Thompson et al., 2006.

Reports provided by scheme provider
The scheme provider will provide a performance report to each participant and show the distribution of results from all laboratories together with participant’s performance score.

The test results as used by the scheme provider will also be available, to enable participants to check that their data have been correctly entered.

Reports will be made available after the return of results to the coordinating laboratory.

References

Ås, 10 April, 2014.
John-Erik Haugen
Nofima Food AS
11. Annex 4: Human nose study protocol

Evaluation of the online performance

This document describes an experimental set-up for a minimal scenario where 300 carcasses are evaluated by the on-line method at industrial level and sampled for evaluation by an expert panel at ILVO. A subgroup of minimum 50 carcasses should be sampled for further evaluation by a second expert panel (at UGo) and chemical analysis (measurement of the levels of androstenone, skatole and indole in fat). Sensitivity and specificity will be measured compared to the ILVO expert panel, but not compared to chemical analysis (as not all samples are evaluated by chemical analysis). For this, a participation fee of 5000 € is demanded for participation by the slaughterhouse.

When evaluating the online methodology, it should be taken into account that:

- Per occasion, evaluation of boar taint is mostly performed by only 1 expert
- This experts mostly score maximum 300 carcasses per occasion
- As boar taint prevalence probably varies between 3 to 10 %, at least 300 carcasses should be included in the performance test
- Several experts are trained to perform this methodology, but not all can be included in the online performance test. Therefore it is not possible to evaluate inter reliability of all experts for the same carcasses
- It is impossible to evaluate intra reliability of one expert online
- A supplementary protocol is provided for at line evaluation of a subgroup of samples in order to evaluate intra reliability of the expert(s) as well as inter reliability of a higher number of experts
- Budget is foreseen to evaluate a number of fat samples by the UGo expert panel
- All fat samples can be evaluated by three ILVO experts
- There is no budget foreseen to evaluate fat samples by chemical analysis, therefore a participation fee will be requested to cover the costs of chemical analysis of 50 samples and shipment costs.

The set-up of the study is shown in Figure 1. Each project partner will follow one slaughter session per slaughterhouse. At that day, the online performance of the boar taint detection method will be evaluated (Step 1). The same day, also at line performance of a larger number of slaughterhouse experts and olfactory acuity of these experts will be tested (Step 2) to compare online and at line performance and to gather information about the performance of a larger number of experts performing the human nose methodology.

All carcasses will be sampled

- for sensory evaluation by at least 3 ILVO experts (Step 3)
- based on these results we will determine the sensitivity and specificity of the online method compared to the ILVO expert panel evaluation.
- All samples for which there is disagreement between the online evaluation of the slaughterhouse experts and the ILVO experts, and which are not yet included in sampling A (subgroup of samples that will be further evaluated, see below), will also be sent to the UGo panel (added to sample A2) (Step 4) and to the lab for chemical analysis (added to sample A3) (Step 5)
- All remaining samples will be stored and can be sent to a lab in case there is budget for chemical analysis of all fat samples (Step 6/optional)
A subgroup of carcasses (n ≥ 50) will be sampled. This subgroup will include all samples with boar taint according to the online evaluation (identified as having boar taint by at least 1 expert) and at least 40 samples without boar taint (Sampling A).

- Sampling A1: 50 samples for the at line evaluation by at least 4 slaughterhouse experts if possible (Step 2)
- Sampling A2: 50 samples for the UGo panel evaluation by 5 to 10 experts (Step 4)
- Sampling A3: 50 samples for chemical analysis (Step 5/mandatory)

The information provided by the UGo panel and the chemical analysis will provide more information on the subgroup of samples and may clarify differences/inconsistencies between the online method results and the ILVO expert panel results. Unless the second optional step 6 is performed, it will not allow the measurement of sensitivity and specificity compared to chemical analyses nor compared to the UGo expert panel.
Step 1 - Online evaluation: Evaluation of samples online by at least two slaughter experts (if possible), scoring at the same time

Aim
- Boar taint scores by at least two experts of at least 300 carcasses, evaluation performed online

Material and methods
- (At least) two slaughterhouse experts score the same carcasses blind(!) and online, according to their routine practices
- Number of evaluated carcasses: \( N \geq 300 \)
- All carcasses should be well numbered!
- Scores are registered by the project partner representatives, and well linked to the carcass number

Sample collection
Methodology
- All carcasses should be sampled according to the figure presented below.
- Samples should be taken from the same sample location from the other opposite carcass (not heated)
• Sensory @ ILVO:
  o For all carcasses, neck fat (of 15 cm x 6 cm x entire fat layer) should be collected and stored in a plastic bag, vacuum packed, well identified, kept frozen (-20°C) and send to ILVO by the slaughterhouse. => step 3

• Sensory @ slaughterhouse (sample A1)
  o A subgroup of carcass will be sampled for sensory @ slaughterhouse => step 2:
    ▪ all carcasses with boar taint (by at least one slaughter house expert, as evaluated online)
    ▪ at least 40 samples without boar taint (more if there are less than 10 samples with boar taint in total).
    • Sampling procedure: sample every 7th carcass. Take the next carcass in case the 7th carcass has boar taint
    => The total number of samples should be at least 50 or more if there are more than 10 samples with boar taint!
  o All remaining (= not selected) samples should be stored, vacuum packed and frozen, by the project partner

• Sensory @ UGo
  o all carcasses should be sampled for sensory @ UGo. The further selection of samples is the same subselection as A1 + all samples with disagreement between slaughterhouse and ILVO (=false negative).

• ASI-analysis
  o all carcasses should be sampled for ASI-analysis. The further selection of samples is the same subselection as A1 + all samples with disagreement between slaughterhouse and ILVO (=false negative).
  o For these selected samples, skin layer should be removed from the fat before storage
  o OR (optional): all samples for ASI-analysis in case costs are covered by the slaughterhouse

General remark: Make sure that the complete fat layer is sampled.

Material needed for sample collection

• If samples are collected after cooling: well identified labelled bags, including number + country
• If samples are also collected online: System to store the samples in correct order. We use this type of labelled boxes (Sortimo). Perhaps the slaughterhouse has it’s own easy-to-use system. Probably sufficient sample material for ILVO and SH experts, but not for UGo. Sample collection online is not necessary if samples can be collected after cooling.

Step 2 – Sensory @ slaughterhouse: Evaluation of a subgroup of samples at line and olfactory test by at least four slaughterhouse experts

Aim:
• Boar taint scores of a selection of (at least) 50 samples
• Evaluated at line by at least 4 experts
  ▪ the two experts who scored boar taint online
  ▪ at least two extra experts
• Expert olfactory acuity test
Material, people and methods

- **Who?**
  - The two slaughterhouse experts (which scored the carcasses online) should score the same samples at line
  - At least two other experts should also score the selected samples at line

- **At line evaluation**
  - At the same scale as performed online if possible and according the methodology used for evaluation at line (if this is the case) or hot iron method if at line method is not yet performed
  - A protocol with random sample order will be provided. This should be followed for all experts.
  - Scores are registered by the project partner/the slaughterhouse expert, and well linked to the sample number

- **Expert olfactory acuity test**
  - All experts will be screened for skatole and androstenone sensitivity according to the UGo paper strip – triangle methodology
    - All triangle tests consist of one odd sample (the respective odorant) and two identical samples (solvent only, i.e. propylene glycol). The first triangle is used to acquaint the participants with the test procedure and comprised of D-carvone (1.5 % in propylene glycol) which has a mint-like odor and is usually perceptible to everyone (Mörlein et al., 2013 learning to smell paper). Thereafter, two different concentrations of androstenone (0.5 µg/g and 5 µg/g) and a single concentration of skatole (1 µg/g) are assessed in triplicate each.
    - Odor liking of androstenone (5 µg/g) and skatole (1 µg/g) will be assessed by again presenting the odorants individually and scored using a 9 point hedonic scales.

**Step 3 - Sensory @ ILVO: Sensory evaluation by the ILVO expert panel of all samples**

**Expected results:**

- Identification of samples with disagreement between slaughterhouse experts and ILVO experts (x)
- Evaluation of online method performance compared to the sensory evaluation of the ILVO experts (sensitivity, specificity)

**Material and methods**

- All samples will be evaluated by at least 3 ILVO experts
- Experts evaluate boar taint on a scale from 0 to 4. Samples are identified as having boar taint if mean ≥ 2.5.
- Results of the slaughterhouse experts and the ILVO experts will be compared and false negative as well as false positive samples will be identified. These samples should be included to the sample group for sensory @ UGo and ASIS-analysis

**Step 4 - Sensory @ UGo: Sensory evaluation of the subgroup of samples by the UGo expert panel**

**Aim:**

- Sensory evaluation of the selected samples + extra samples in case samples were identified as false negative or false positive by the ILVO panel
Material and methods
- The samples will be sent to UGo by the slaughterhouse (or costs covered by the slaughterhouse)
- Expert panel evaluation will be performed by at least 5 assessors per sample, all samples will be presented in randomized order between panelists.
- Samples are evaluated for deviation from standard back fat odour, intensity of skatole odour, intensity of androstenone odour and intensity of other off-odours not identifiable as skatole or androstenone
- Samples are scored on a scale from 0 to 5. Samples are identified as having boar taint if mean ≥ 2.5.

Step 5 - Laboratory analysis of the subgroup of samples (Mandatory)
Results:
- Chemical analysis of indole, skatole and androstenone of the selected samples + extra samples in case samples were identified as false negative/positive by the ILVO panel

Material and methods
- The samples will be sent to the selected lab by the slaughterhouse (or costs covered by the slaughterhouse)
- Samples will be analysed for indole, skatole and androstenone content by the selected lab

Step 6 - Laboratory analysis of all samples (Optional)
Results:
- Chemical analysis of indole, skatole and androstenone of all remaining samples

Material and methods
- The collected samples that are not yet analysed will be sent to the selected laboratory by the slaughterhouse (or costs covered by the slaughterhouse)
- Samples will be analysed for indole, skatole and androstenone content by the selected lab

Evaluation of at line performance - intra and inter reliability

The first step when evaluating this sensory method, is to determine the intra and inter reliability of the slaughterhouse experts. This evaluation is performed at line. The results will be compared with the sensory evaluation of the samples by two expert panels (UGo and ILVO).

Three replicates of 30 samples (20% boar taint) are presented blind and in random order per repatentment and per expert (see schedule). So experts will evaluate 90 samples in total, with two times a 10 minute break between the 30 samples. Based on this experimental set-up, we can determine the intraclass correlation coefficient, intra and inter reliability.

<table>
<thead>
<tr>
<th>First replicate</th>
<th>Second replicate</th>
<th>Third replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 samples</td>
<td>Same 30 samples</td>
<td>30 samples</td>
</tr>
<tr>
<td>20% boar taint</td>
<td>20% boar taint</td>
<td>20% boar taint</td>
</tr>
<tr>
<td></td>
<td>(Different order)</td>
<td>(Different order)</td>
</tr>
</tbody>
</table>
All experts will be screened for skatole and androstenone sensitivity according to the UGo paper strip– triangle methodology. All triangle tests consist of one odd sample (the respective odorant) and two identical samples (solvent only, i.e. propylene glycol). The first triangle is used to acquaint the participants with the test procedure and comprised of D-carvone (1.5 % in propylene glycol) which has a mint-like odor and is usually perceptible to everyone (Mörlein et al., 2013 learning to smell paper). Thereafter, two different concentrations of androstenone (0.5 µg/g and 5 µg/g ) and a single concentration of skatole (1 µg/g) are assessed in triplicate each.

Odor liking of androstenone (5 µg/g) and skatole (1 µg/g) will be assessed by again presenting the odorants individually and scored using a 9 point hedonic scales.

The fat samples and the paper strips will be send by ILVO to the project partner of the country involved. The tests will be performed under supervision of this person. Scores will be recorded and reported by the project partner in cooperation with the participant. At least two, preferably three experts per slaughterhouse should participate in this test.

Fat samples should be evaluated according to routine practice for at line evaluation. Results should be presented according to routine practice: scale or positive/negative and according the extra scale provided by the project partner.

The participants should include a brief outline of the analysis protocol(s) being used comprising both total number of assessors, number of assessors involved in the test, definition of boar taint (boar taint /deviating odor and scaling).