MYCOHUNT

Rapid Biosensor for the Detection of Mycotoxin in Wheat

Report on the biological and technical specifications of the MYCOHUNT system

D1.1. Report

PUBLIC

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Table of Content

1. Public summary

In this document the D1.1 Deliverable MYCOHUNT project is summarized. The aim of the project is to develop a cost-effective method to detect infection of mycotoxin deoxynivalenol in wheat grains. In this deliverable we summarize the system specification. First, the corresponding European regulations and current methods are summarized; they provide the essential background information for market analyses. The market need was examined from two sites. On one hand a Questionnaire was prepared, sent to partners and translated to languages of the consortium members. The answers are collected continuously; the first results are summarized in this Deliverable. The results of questionnaire survey will be continuously up-dated on the confidential project web page. On the other hand, the market need is analyzed based on the experience of consortium members and on available public information. The technical specification of the MYCOHUNT device is also provided in details herein.

2. Abstract of the Project

The Mycohunt project aims at increasing the competitiveness of a large group of SMEs by developing a cost-effective method to detect infection of mycotoxin deoxynivalenol in wheat grains, a major threat to the food and feed sector of the European industry. A group of SME-AGs, covering the two relevant sectors and representing vast number of sector SMEs, have put together this project in order to gain knowledge and resources to further exploit the results of the novel technology proposed by providing a thorough sampling and measurement method of grain.

The economic consequences of mycotoxin infection to animal husbandry and the cost of infected crop to farmers as well as mill and storage house owners are of increasing concern while human health and safety effects are and must be addressed additionally.

3. Objective of this Deliverable

The first of the task of this deliverable is to discover market situation and the needs of different stake holders. Preparation and distribution of questionnaires in the different European languages is aimed. According to the description of work (Annex I, DOW), The objective is to obtain information regarding:

- currently used sampling and detection methods for DON

- degree of satisfaction with these methods and needs not covered by them

- required speed and sensitivity for DON detection

- price sensitivity

- legal and regulatory issues for implementation purposes

Based on the information gathered and on the technical feasibility and experience of the partners involved in the project, the system technical specifications is aimed to be given in this deliverable.

4. Regulations and current methods

In order to understand the market needs, first the regulation and the methods currently applied were summarized by the consortium .

4.1 European regulations

The toxins produced by Fusarium species are of particular significance in agriculture. A major class of Fusarium toxins are the trichothecenes, which can be found in cereals. Over 150 trichothecenes are known, of which deoxynivalenol (DON) is the most well known. DON leads to undesirable effects on the immune system, to growth retardation in children and to feed refusal by pigs.

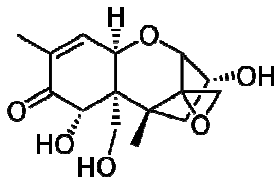


Figure 1 DON structure

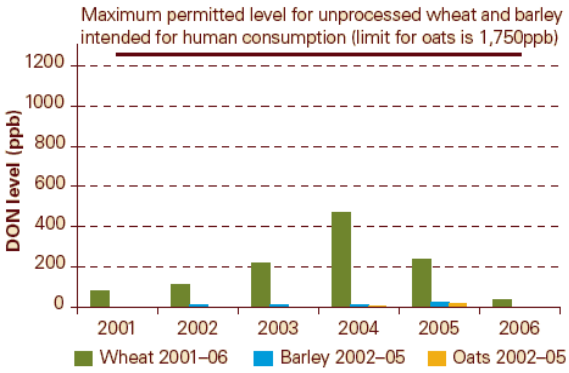


Figure 2: Maximum permitted level of DON

Commission Regulation (EC) No **466/2001** of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs has been substantially amended many times. It is necessary to continuously amend EU policies intended to reduce human exposure, crop contamination, given the potential economic loss for producers, marketers, and processors of diverse susceptible crops.

The analytical methodology (EIA) will be developed in a way to accommodate with the expected EU legal limits for the DON which in case of unprocessed cereals other than durum wheat, oats and maize is 1250 µg/kg (maximum level) as required by the Regulation **1881/2006 EC**.

Having in consideration the expansion in the European mycotoxin regulatory activity there is a need for new methods, which are faster and cheaper than the present ones. The current analytical methods used for the detection of DON are from grains (wheat kernel) and are based on laboratory detections as the chromatographic procedures, which are relatively time-consuming and costly. There is a need on for high-throughput, low-cost method that can be widely applicable and which we are preparing in the Mycohunt project. Having in consideration that mycotoxins in the grain dust are the same that are commonly found in grain and the positive correlation between the grain infection and dust infection we intend to determine the DON toxins from the grain dust (airborne) with a fast ELISA immuno assay (EIA) antibody-based electrochemical (amperometric) biosensors.

4.2 Current sampling methods

Usually the lab analyst has no influence on the sampling and gene-rally the received sample is simply ground in the laboratory by a small laboratory mill. Several studies of the sampling variability of mycotoxins have been published [1], sampling plans are available for Fusarium toxins in different commodities [2] but they differ country by country. A mycotoxin-sampling plan is defined by the mycotoxin test procedure (sample size, sample preparation method, and analytical method) and the accept/reject limit. Because of the variability associated with each step of the mycotoxin test procedure, the true mycotoxin concentration of a bulk lot cannot be determined with 100% certainity. As a result, the sampling program will misclassify some lots. It is important to understand the sources of error in the mycotoxin test procedure so the errors can be effectively reduced. The sampling step usually is the largest source of error due to the extreme mycotoxin distribution among kernels in the lot [3].

To ensure the reliability of measured levels of mycotoxin it is important to follow a well established sampling procedure. A wrong sampling plan affects the results, giving rise to discharge of sound cereal products or in the other way accept contaminated products as the contamination is not detected during intake. In both cases there is an economic loss and it can result in legal disputes and barriers to trade.

European regulations establish the procedures for the sampling of bulk and retail products potentially contaminated by mycotoxins [4]. This EC regulation gives practical guidelines on how to draw samples. The regulation states that sampling plays a crucial part in the precision of the determination of the levels of mycotoxins considering that they are very heterogeneously distributed in a lot. It is therefore necessary to fix general criteria which the sampling method should comply with.

Table 1: Subdivision of lots into sublets depending on product and lot weight

Commodity	Lot weight (tonnes)	Weight or number of sublots	Number of incremental samples	Aggregate sample weight (kg)
Cereals and cereal products	≥ 1500	500 tonnes	100	10
	> 300 and < 1500	3 sublots	100	10
	≥ 50 and ≤ 300	100 tonnes	100	10
	< 50	-	3-100 (*)	1-10
(*) Depending on the lot weight - see Table 2.				

Following the EC official method in Table 1 and 2 this means that for each vehicle carrying approximate 30 tons of cereals (for example delivery from grower to trader) a number of 100 incremental samples should be taken upon deliverance and the aggregate sample should be 10 kg. This amount of samples withdrawn upon delivery is that high that at cereal traders level sampling will differ a lot from the official method. Taking into account that the (official) method described in the regulation is based on the heterogeneously distribution of mycotoxins in a lot, the sampling methods at trader level should be representative for the lot of cereals to be sampled. For the non-official sampling, it is important to have a method which is easier to implement but without loss on reliability.

Table 2: Number of incremental samples to be taken depending on the weight of lot of cereals and cereal products

Lot weight (tonnes)	Number of incremental samples	Aggregate sample weight (kg)
≤0,05	3	1
>0,05 and ≤ 0,5	5	1
>0,5 and ≤ 1	10	1
>1 and ≤ 3	20	2
>3 and ≤ 10	40	4
>10 and ≤ 20	60	6
>20 and ≤ 50	100	10

Some national guidelines in EU-27 differ from the EC recommendation, according to good manufacturing practices, they describe statistically representative sampling guidelines for bulk cereals. On delivery 20 samples from the entire delivery of bulk cereals during unloading should be taken. Sampling is carried out with a device cutting the stream during unloading, which will lead to 10- 25 kg sample, well mixed. In a bin one should take 12-20 deep probe samples, including samples from the sides of bins or edges of storage where mold is likely to occur. A composite sample for submission to the lab should be 500 g.

Despite of the existing sampling methods, in practice due to a lack of time during delivery and the reception of the cereals, sampling is often carried out in a non representative way, which may lead to incorrect results and decisions.

4.3 **Current analytical methods**

Current methods used for DON determination include chromatographic methods such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC) with electron capture detection, mass spectrometric (MS), or fluorescence detection. Although sensitive and accurate, most of the chromatographic methods developed are laborious, expensive, time-consuming, and have low throughput.

4.3.1 The standard HPLC test procedure

The performed DON analysis takes in account the requirements in the DON standard analysis according to **CEN standard 15791:2009**, Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding and Commisssion Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

Extraction of DON from the sample

Transfer 25.0 g of test material (baby food or animal feed) into a 250 or 500 mL conical flask. Add 200 mL of deionised water, cap and shake for 1 hour on a bench top shaker.

Clean-up

Prepare a funnel and filter paper. Pour the extracted sample into a 250 or 500 mL conical flask through the prepared funnel and filter paper. Transfer 2 ml of the aqueous layer plus 8 mL of deionised water into the reservoir an immunoaffinity column. Allow this solution to pass slowly through the column. When the extract has passed completely through the immunoaffinity column, pass 5 mL of deionised water. Dry the column by passing nitrogen through the column for about 5 seconds. Then discard all the eluent from this stage of the clean-up procedure. Finally, place a 2.0-2.5 mL HPLC vial under the column and pass 0.5 mL of methanol through the column, collecting the eluate. After the last drops of methanol have passed through the column allow the methanol to remain on the column for approximately 1 minute. Then add a further 1.0 mL of methanol and continue to collect the eluate. Carefully pass air through the column in order to collect any final drops.

Preparation of test sample for HPLC analysis

Evaporate the eluate collected from the immunoaffinity column to dryness at 35℃ under nitrogen. Immediately cool the HPLC vial to ambient temperature and re-dissolve the residue in a final volume of 0.500 mL mobile phase. Crimp the HPLC vial and make sure that the residue is completely re-dissolved by shaking for at least 30 seconds. If necessary the sample may be filtered before analysis by HPLC. A check should be made with a standard solution to assess any loss of DON due to this filtration step.

Spike recovery

Using a micropipette or preferably a Hamilton syringe add 1000 µL of the spike recovery solution to 25 g of blank matrix, allow to stand for 1 hour and extract as described above. A spike recovery should be carried out with each analytical batch.

If necessary wash the HPLC system thoroughly with 100 % methanol after each sample injection to ensure that there are no materials retained on the column.

Preparation of the calibration graph

Prepare the calibration graph by injecting a full loop (100-300 µL) of at least five standard solutions of different suitable concentrations into the chromatograph. Plot the peak area (or height) values of the DON calibration solutions against the concentration in µg/kg of DON in the sample.

Determination of DON in test solutions

Inject aliquots of the test solutions into the chromatograph using the same conditions used for the preparation of the calibration graph. Identify the deoxynivalenol peak of the test solution by comparing the retention time of the sample with that of the calibrants.

Calculation

Quantitative determination is carried out by the integration of the peak area (or peak height). Determine the content of deoxynivalenol in the test material, in µg/kg, directly from the calibration graph

Calculate the concentration of DON in the test sample as follows (copied from CEN standard 15791:2009):

Calculation of the calibration curve (function) obtained by linear regression:

$$C_{smp}\ [ng/mL] = a \times Signal_{smp}\ [units] + b$$

Further calculate the contamination level of DON in the test material according to:

$$DON\ [ng/g] = \frac{Csmp \times Solvent \times Elution}{W \times Aliquot} \left[\frac{ng \times mL \times mL}{mL \times g \times mL} \right]$$

$$DON\ [ng/g] = Csmp \times 0.4 \text{ (for baby food)}$$

$$DON\ [ng/g] = Csmp \times 2 \text{ (for animal feed)}$$

Plot the data - concentration of DON [ng/mL] (y-axis) against the peak signal (x-axis)- from the calibration experiments into a table and calculate the calibration curve using linear regression. Use the resulting function (y = ax + b) to calculate the concentration of DON in the measured solution (where a is the value of the slope of the linear function and b is the value where the calibration function intercepts the y-axis of the co-ordinate system).

W (g) = sample material taken for analysis

Solvent (mL) = solvent taken for extraction

Aliquot(mL) = aliquot taken from extract for immunoaffinity clean-up

Elution (mL) = final volume achieved after elution from IAC

Csmp (ng/mL) = concentration of DON in the injected solution calculated from linear regression

Conta. (ng/g) = contamination of sample material with DON

Signalsmp = signal of DON peak obtained from the measured solution

HPLC column and conditions

Octadecylsilane (ODS) – Fully End Capped with

- a length of 250 mm
- an internal diameter of 4.6 mm
- stationary phase with particles of size 3 µm, 80 Å
- carbon loading of 11.5 %

Precolumn – ODS with

- a length of 10 mm
- an internal diameter of 4.0 mm
- stationary phase with particles of size 3 µm

Mobile Phase - water : methanol : acetic acid (85+15+0.1 [v/v/v])

Flow rate – 1.0 mL/min

Injection volume – 200 µL

Detection – UV detector set at 220 nm

5. Market needs

5.1 **Market search**

In order to understand the market needs we have prepared a questionnaire: see in the Appendix. The questionnaire was translated to different languages by the members of the consortium: Spanish, French, Hungarian, Polish, Greek, Dutch.

The questionnaire was sent out to partners of the members of the consortium. Also companies around Europe were asked to fill in the forms. Due to the slow response time, the answers could not be fully validated by this time, and the results will be continuously updating on the webpage and discussed by the partners at general meetings.

On the other hand, we can already get the following consequences for the Market search (from about 50 responses):

1. There are three different groups interested in the MYCOHUNT system:

   a. Traders, who would like to ensure that no mycotoxin contamination occurred. This group would not like to pay more than 5000 (maximum 10.000) Euros for MYCOHUNT device. They currently carry out analysis out sourced. For this group performance and price are the most important features.

   b. Milling and food producer. They would like to pay more for the analysis, but for them reliability is the most important issue.

   c. Authorities are also interested in MYCOHUNT system (5 from 50). From their point of view only reliability matters the most, but performance is also important.

2. The companies participated in filling the questionnaire did not report any DON contamination, or they did not want to declare it in such questionnaire.
3. Dilution (mixing with low contaminated grain) is the most common procedure, farmers apply, when DON contamination occurs.
4. Apart from authorities, most of the companies carry out analysis externally (45/50), however the sampling is carried out both in house (22/50) and external company (13/50).

The consortium will make all effort to give a more detailed response rate and to give detailed analysis. It will be discussed by the partners, and results will be continually updated on the web-page.

5.2 **Market analysis**

Based on the responses gained so far and the literature data, the following market analysis can be given. Arable crops cover 40% of the European Union's utilized agricultural area and are an essential aspect of the economy of all Member States. According to recent Europe statistics, total available wheat stocks from Jan-Jun 2008 represented 151 million tons, of which 46.3 million were for human use [5]. The total value of agriculture production of EU-27 was 308,023 million Euros in 2006, with cereals alone accounting for over 30,630 million Euros, and livestock production, which is highly dependent on feed production, accounting for 131,124 million Euros [6]. Cereals represent almost 11% of the shares of individual products in agricultural output and cattle and pigs approximately 16% and 9%, respectively [7]. The level of fusarium mycotoxin varies widely by cultivates and strongly depends on weather conditions. Sharp increases and decreases in crop price and volume are a reality within the agricultural sector, and have a significant impact on small farms and SMEs in agriculturally-related businesses. While agriculture represents a tremendous economic foundation for the European economy, in the cereals sector, internal prices are, on average, still higher than world prices. This makes it very hard to export European cereal crops, and

products processed from them, without the use of government subsidies. This fact, together with variations in prices, has a dramatic effect on the millions of small farmers who have less capital to overcome the ups and downs of the market. Improved technology such as MycoHunt will aid farmers in those years when the harvest is low, by salvaging and protecting uncontaminated grain, maximizing output, increasing volume and, consequently, profits. Accurate identification of mycotoxins will benefit the EU as a whole, improving the overall quality of wheat and, in doing so, increasing EU competitiveness in the world cereal market.

The economic relevance of the MycoHunt technology to the EU agricultural sector overall, and to small farmers in particular, is that it will

- ensure high quality grain production without significant loss of load weight due to contamination
- increase sales profits resulting from high quality grain which costs for
  - human consumption: 173-192€/t in the national markets or 190- 200€/t to export
  - animal feed: 130-144€/t in national markets or 140-150€ to export (prices of August 2008)
- save money by early prevention of DON inspection with regular monitoring
- avoid cleaning costs of contained grains (an average €0.28/bushel of wheat charge)
- avoid penalty fees of fringing the ratio set out by the national authorities

Use of this proposed system will also result in improved germination rates, seedling vigour and grain quality and more accurate identification of contamination. Mycotoxins cause spoilage and quality deterioration in crops, reduced health of livestock from the use of contaminated feed, and may cause adverse health effects for humans who consume contaminated food. The economic losses associated with mycotoxin contamination are difficult to assess, and no comprehensive analysis of the costs to crop/livestock producers is available. However, with an estimated 25 to 50% of the world's food crops affected by mycotoxins, the economic costs are likely to be considerable [8]. For example, the Council for Agricultural Science and Technology estimated that crop losses from mycotoxin contamination in the United States amount to $932 million annually, in addition to losses averaging $466 million annually from regulatory enforcement, testing, and other quality control measures.

The combined effects of the feed and food losses reduce the supply and raise the price of various farm commodities. Improved technology such as MycoHunt will aid farmers in delivering controlled quality product, warehouse owners in monitoring the quality of incoming and outgoing grains. Accurate identification of mycotoxins will benefit the EU as a whole, improving the overall quality of wheat and, in doing so, increasing EU competitiveness in the world cereal market. We assume that due to MycoHunt technology €6 million added value can be achieved at European level over a five-year timeframe after the duration of the project. Consequently, at European level by assuming a 4.5% market penetration of MycoHunt commercialization and adding the economic impact of the project in amount of 2.89 million € with calculating with a 1.015 million initial investment from the EC, the project results in a 100:900 ROI for the EC after 5 years after project completion.

The end-user market of MycoHunt technology consists of 137,004,000 agricultural holdings upon the Eurostat data as of 2007 [7] which are single units both technically and economically, which has single management and which produces agricultural products. The data of Eurostat [9] referring to last years show that the number of agricultural holdings in Europe decreases smoothly (from 150,210,300 in 2003 to 137,004,000 by 2007) which indicates 2% decline as average per year. Nonetheless, the potential market for MycoHunt is still very promising including 9.3 M holdings by 2015.

6. Technical specification

6.1 Concept specification

The aim of the MYCOHUNT project is to use continuous sampling during unload procedure, instead of representative sampling by staff. The concept of them MYCOHUNT project can be visualized in comparison with standard procedure applied today (Figure 3 and 4).

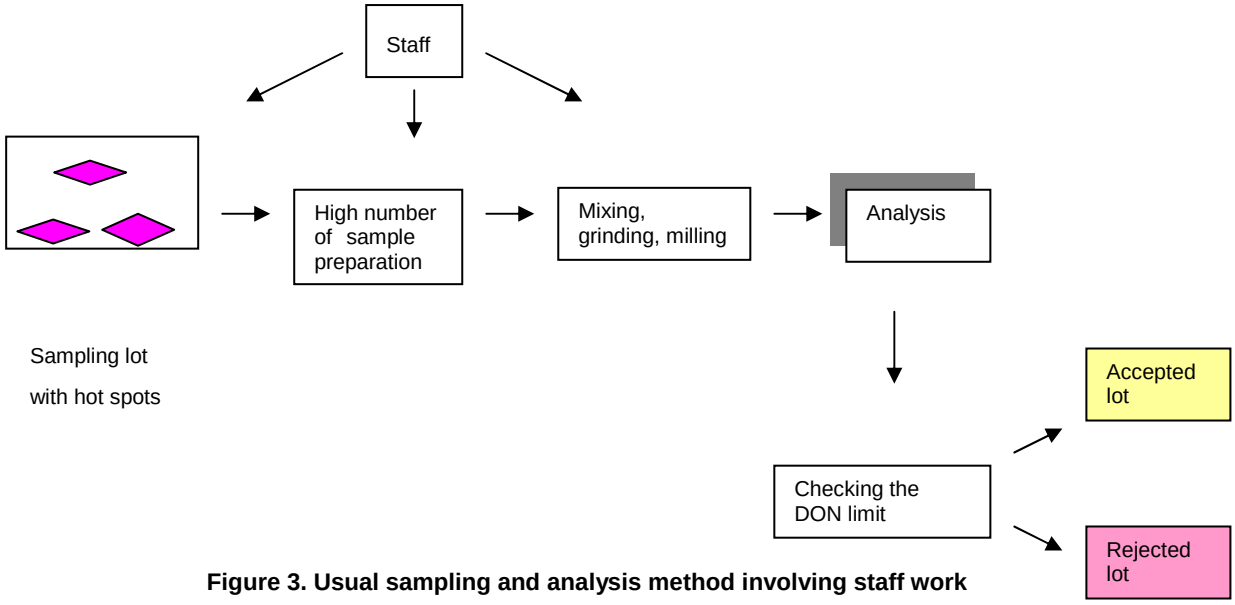


Figure 3. Usual sampling and analysis method involving staff work

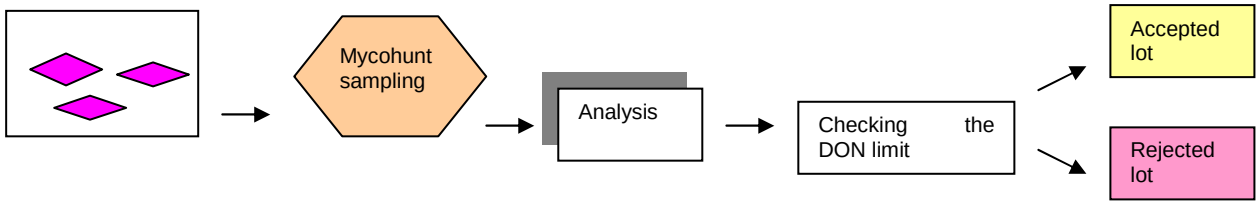


Figure 4. Mycohunt automated sampling method

The technological objectives are the following:

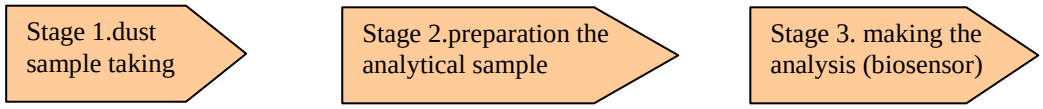
- To develop effective, non-destructive sampling apparatus at max. 4000 € cost in order to assure adequately representative measurement aiming at 90% correlation with the bulk samples.
- To assess detection limit of 50 ppb, efficacy, specificity and sensitivity of the sensor
- To develop a data evaluation algorithms and to set up correlation databases
- To develop the 'plug & play' disposable cartridge to house the antibody-binding assay as well as the portable biosensor analyser suitable for industrial operations.

The scientific objectives:

- The study of the airborne DON and its correlation with the DON found in the grain.
- The understanding and determination of parameters (temperature, pressure, vacuum, etc) affecting the sampling precision avoiding the damaging of grain.

- To develop immobilising methods for sensitive antibodies, and assess different approaches to enhance sensitivity, reproducibility, life-time, detection range, detection limit, and/or other specifications.
- The investigation of the cross-reactivity of other trichothecenes such as 3-acetyl-DON, 15-acetyl-DON, nivalenol, etc.

The proposed MYCIOHUNT method includes 3 stages:



6.2 Specification of the sampling device

6.2.1 Option I: Dust separation by cyclone

The possible scheme of realization of the MYCOHUNT system is shown in Figure 5.

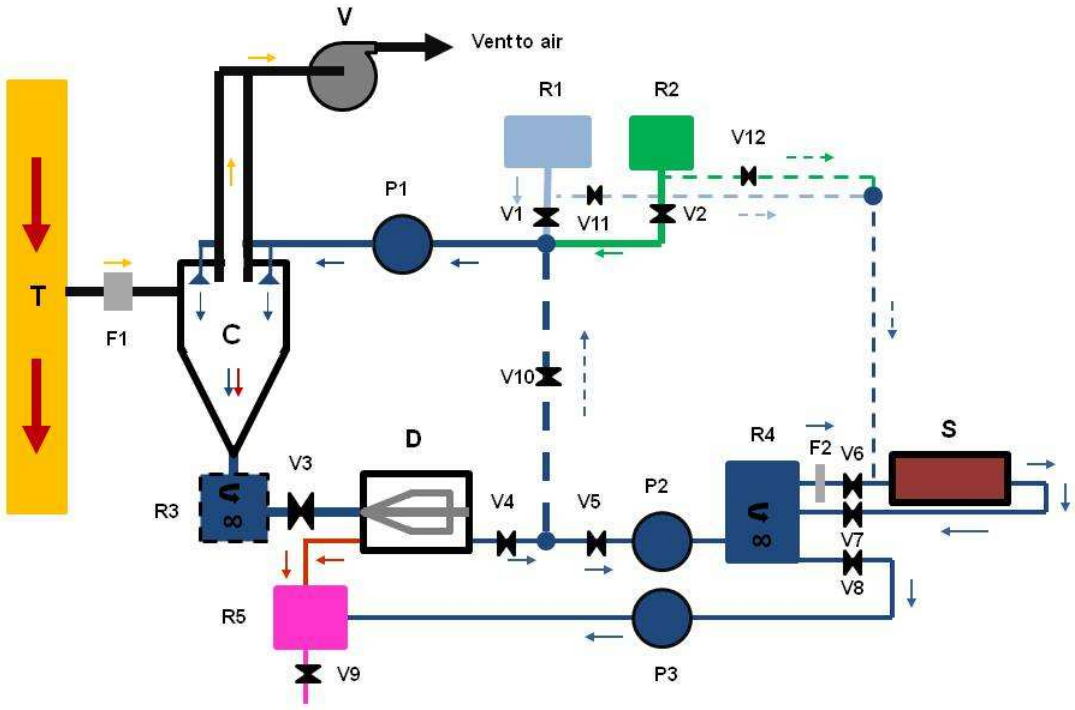


Figure 5. Mycohunt sampling system (option I, with cyclone). Parts signed with broken are lines optional. Abbreviations: T: Loading tube, F1, F2: filters: C: cyclone, V: ventilator, P1-P3: liquid pumps, R1: reservoir of solvent (water/acetonitrile), R2: reservoir for buffering agent (PBS), R3: Optional buffering and mixing reservoir, R4: Buffering and mixing reservoir, R5: waste reservoir, V1-V9 micro controlled valves, S: sensor.

The grain (wheat) dust is taken from the loading tubes (**T**) (usually with a diameter of 25-35 cm) by a ventilator (**V**) with a capacity of 2-10 l/min. The air is pulled in a cyclone (**C**) for the sedimentation of dust fractions. The outlet of the ventilator (**V**) is opened to air. The **F1** filter ensures that no large particles and wheat pass through towards the cyclone.

(Differing from the original plant described in DOW, no additional cyclone is required, because the amount of dust for the DON measurement was only 0.05% of the original wheat, see **D2.1.**)

To generate the washing curtain in the cyclone, a high pressure pump (**P1**) will be used. The size and type of the pump will be designed based on the calculated requirements, and will be

optimized during experimental validation. The washing agent will include two components: **R1** is the washing agent - accordingly to the literature [10-14], acetonitrile,/water mixture (20/80-80/20) will be the most appropriate and **R2** phosphate buffered salin to adjust pH to be around 7. The bottom of the cyclone may be connected to a buffering reservoir (**R3**), in which the dissolution of DON to the solvent will be completed. This device will increase the residence time, but may not be necessary. The received colloidal (dust/solvent) fluid will pass through a decanter (**D**), in which dust and liquid separates. The solid dust would exit to a waste reservoir (**R5**), while the liquid is transported through **V4**. If the concentration in the liquid is not enough, the solvent may be re-circulated, through **V10**, in this case **V5** would remain closed and **P1** pump would transport the solution.

The solution is transported by pump **P2**, to a mixing reservoir (**R4**), in which the solution would be homogenized when all valves **V6**-**V8** are closed. The sample is transported to the bio-imunoassay sensor (**S**) – with a built in pomp – through **F2** filter and **V6** valve. The sample may be re-circulated through **V7**, if necessary. In the case the sample is too concentrated, and/or the pH must be re-adjusted before the sensor, the washing agent and the buffer solution may be added before the sensor (**S**), by **V11** and **V12**, respectively.

The liquid from **R4** is transported by **P3** pump to the waste tank (**R5**).

The washing of the system can be made by by the solvent (**R1**), operating **P1** pump, with all valves open.

6.2.2 Option II: Dust separation by washing flask

The consortium take into consideration the use of a washing flask, instead of the cyclone. The schematic view of this setup is shown in Figure 6.

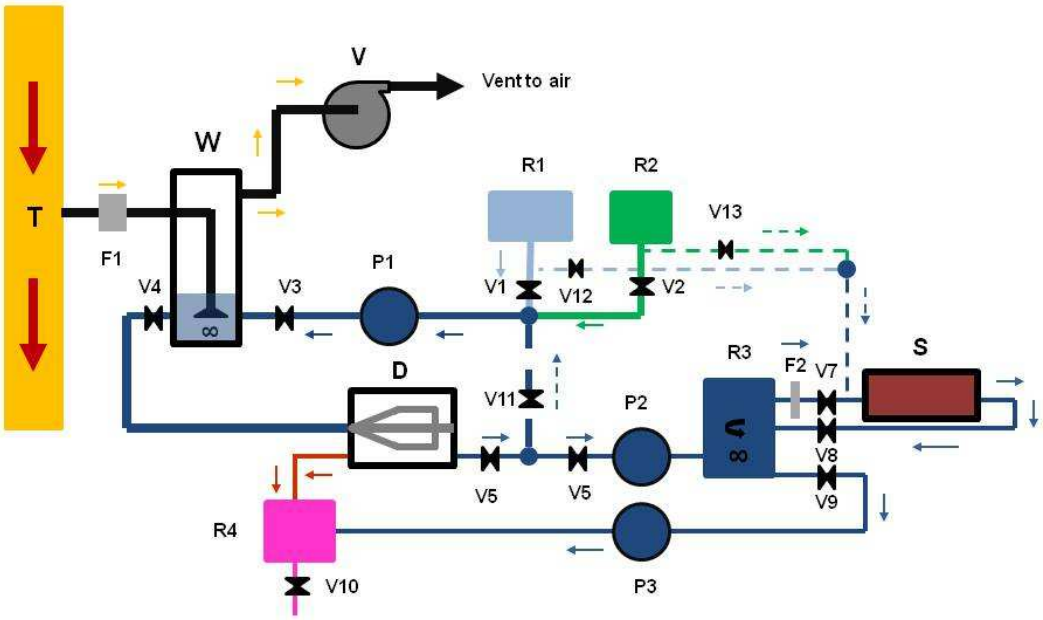


Figure 6. Mycohunt sampling system (option II, with washing flask). Parts signed with broken are lines optional. Abbreviations: T: Loading tube, F1, F2: filters: W: washing falsk, V: ventilator, P1-P3: liquid pumps, R1: reservoir of solvent (water/acetonitrile), R2: reservoir for buffering agent (PBS), R4: Buffering and mixing reservoir, R4: waste reservoir, V1-V10 micro controlled valves, S: sensor.

The system is similar to option I, the only difference, that instead of a cyclone (**C** in Figure 5), a washing flask (**W** in Figure 6) is used. This device will also act as washing mixing and washing tank, and the residence time can be controlled by valves **V3** and **V4**.

6.3 Specification of the sensor

For the biosensor, a small cell would be used. Smaller the sample loop the better the sensitivity.

The minimum level of detection would be 5 ppb. Though depends on Antibody specificity obtained, there are several parameters that can be varied like Ab loading/capacity of column and with sensitive current measurement.

The highest measurement frequency would be limited by the cycle time for each measurement, most probably maximum of 20-30 minutes per measurement is achievable.

Sensor calibration: DON standards (two concentrations in measurement range) would be used to calibrate the sensor. The stability of the sensor decides the frequency range. Twice a day calibration should sufficient. Software would have options for the calibration.

Flow rate for the sensor would be 1 to 1.5 ml/min through the FIA/SIA system.

The detection will be Electrochemical – Amperometric.

The system would employ a multi-selection valve for SIA analysis. The sample made available after the extraction step can be aspirated through of one of the ports.

6.4 Correlation factors to be determined

The correlation between concentration in the wheat and the concentration in the solution can be given as follows:

$$c = \frac{c_w \cdot M \cdot f \cdot \eta_D \cdot \eta_E}{((V_w + V_P) \cdot s + V_D) \cdot \rho} \cdot 10^{-9}$$

where:

c: is the concentration measured by the sensor in ppb

c_w: is the mycotoxin concentration in wheat in ppb, i.e. mg/tones

M: mass of wheat loaded in tones

f: fraction of dust in the wheat loaded in % (0.05% according to the measurements described **D2.1**)

η_D: dust concentration efficiency, i.e. ratio of mycotoxin concentration in the dust and wheat in %.

η_E: extraction efficiency, i.e. the ratio of mycotoxin concentration in the extract and in the dust, in %.

V_W: is the volume of washing agent added to the M mass of wheat (in l)

V_P: is the volume of buffer added to the M mass of wheat (in l)

s: is the ratio of sampling, i.e. 0.01 means, that 1 ml sample is taken for measurement from 100 ml

V_D: is the volume of diluting agent added (in l)

ρ: is the density of the solution in kg/l. It can be considered as 1 kg/l.

The parameters shown in bold will be determined by laboratory measurements and development phases in WP2-WP5.

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8. Appendix: Questionnaire

(the questionnaire is also available on-line at www.mycohunt.eu)

Dear Sir or Madame,

We are working on a research project funded by the European Commission intended to improve the competitiveness of small and medium enterprises (SME). The aim of the MYCOHUNT project is to develop a cost-effective method to detect infection of the mycotoxin deoxynivalenol (DON) in wheat grains. The goal of the MYCOHUNT project would be twofold: a new sampling technique guaranteeing a 95% bulk transparency and a biosensor technology for the detection of deoxynivalenol.

Since the main principle of this project is to provide a system that acts on the needs of SMEs, we would appreciate if you could share with us your point of view about DON contamination and its detection. We are interested in the current sampling methods that your company applies and the desired developments if there is any. This information will enable us to make a new design closer to your real needs.

Please find below the mentioned questionnaire and take a few moments to fill out this short survey. You can send it back to us at your earliest convenience by e-mail, fax or mail (for addresses see below).

Thank you for taking the time to answer these questions.

Should you need any further information about the above subject, please do not hesitate to contact us.

E-mail:

mycohunt@mfkk.hu

Tel: +36 1 787 4024

Fax: +36 1 787 4390

Mail Address:

MYCOHUNT survey

Budapest, Tétényi út 93.

1119

Hungary

We look forward to hearing from you soon.

Kind regards,

Dr. Wootsch Attila,

Technical Project Coordinator

Questionnaire

1. Please choose your business activity from the below list:
   - ☐ Grain producer
   - ☐ Industrial milling
   - ☐ Feed producer
   - ☐ Grain storage/processor
   - ☐ Trader
   - ☐ Other (Please specify) _____

2. Please give the number of your employees:
   - ☐ Fewer than 10
   - ☐ Between 10 and 50
   - ☐ Between 50 and 250
   - ☐ More than 250

3. Please confirm that your annual turnover is less than 50 million € and/or balance sheet total less than 43 million €
   - ☐ yes
   - ☐ no

4. Please specify the amount of grain produced/processed/distributed per year
   - ☐ Less than 10.000 tons
   - ☐ 10-25.000 tons
   - ☐ 25-50.000 tons
   - ☐ 50-250.000 tons
   - ☐ More than 250.000 tons

5. In which country(ies) is your company located?

   _____

6. Are the following mycotoxin related regulations relevant in your activity?

	food	feed
Prevention (2006/583 EC)	☐	☐
Maximum DON limits:	☐	☐
(1881/2006, 1126/2007, 576/2006 EC)		
Official control (882/2004 EC)	☐	☐
Sampling (2006/401)	☐	☐
Other Mycotoxin related (e.g. National)	☐	☐

7. Does your company apply food and feed safety systems? e.g. GMP

   ☐ No

   ☐ Yes Specify system: _____ Since when?_____

8. Are you aware of mycotoxin contamination? Please indicate: 0: not at all, 5 considered seriously

   0 1 2 3 4 5

9. If you are a grain producer what preventive measures do you take against mycotoxin contamination?

   ☐ Crop rotation

   ☐ Growing mold-resistant grains

   ☐ Using pesticides

   ☐ Paying for expert advice

   ☐ Other

   ☐ Not relevant for my organization

10. If you are a grain trader/processor or feed producer what measures do you take to prevent mycotoxin contamination?

    ☐ Temperature control and registering

    ☐ Air humidity control and registering

    ☐ Regular cleaning of storage and vending locations

    ☐ Dilution

    ☐ Complying with the GMP/ HACCP methods

    ☐ Other

    ☐ Not relevant for my organization

11. Which mycotoxins does your company test and how often?

	Not tested	Monthly	Quarterly	Every half a year	Annually
Aflatoxin	☐	☐	☐	☐	☐
DON	☐	☐	☐	☐	☐
OTA	☐	☐	☐	☐	☐
Zearalenon	☐	☐	☐	☐	☐
Fumonizin	☐	☐	☐	☐	☐
T2/TH2	☐	☐	☐	☐	☐
Ergot alkaloids	☐	☐	☐	☐	☐
Other:_____	☐	☐	☐	☐	☐

if tested:

12. Which mycotoxins do you find most often? Please indicate!

	never	rare	sometimes	often	very often
Aflatoxin	☐	☐	☐	☐	☐
DON	☐	☐	☐	☐	☐
OTA	☐	☐	☐	☐	☐
Zearalenone	☐	☐	☐	☐	☐
Fumonisin	☐	☐	☐	☐	☐
T2/TH2	☐	☐	☐	☐	☐
Ergot alkaloids	☐	☐	☐	☐	☐
Other:_____	☐	☐	☐	☐	☐

if you carry out tests:

13. Can you indicate the yearly amount of money spent for analysis of mycotoxins (sampling and test)?

Annual cost of sampling and sample storage of samples for mycotoxin analysis:_____ €

Annual cost of analysis of mycotoxins:_____ €

14. Could you quantify financial losses due to mycotoxin contamination in the past 5 years?

- ☐ yes, the average loss was _____€/year in the last 5 years
- ☐ no, we had financial losses due to mycotoxin contamination, but I cannot quantify how much
- ☐ No financial loss in the last 5 years due to mycotoxin contamination

Sampling

15. When is sampling for DON tests performed?

- ☐ Before/on harvest
- ☐ At intake/reception
- ☐ During storage
- ☐ Before discharge/distribution/processing
- ☐ Final product
- ☐ Never

16. If sampling for DON is carried out, who does the sampling?

- ☐ In-house
- ☐ External company
- ☐ Authority

17. Which sampling methods do you use?

- ☐ Manual
- ☐ Automated
- ☐ Please specify_____

Analysis

18. If DON analysis is carried out, who does it?

- ☐ In-house
- ☐ External company
- ☐ Authority

19. What methods does your company use for DON detection?

- ☐ Qualitative rapid testing method (yes/no-test)
- ☐ Quantitative ELISA
- ☐ Quantitative HPLC/TLC
- ☐ Other

20. Have you participated in any international or local action/monitoring for the measurement of DON contamination in a particular region in the last 5 years?

- ☐ No
- ☐ Yes
- ☐ If yes, please specify which _____

_____

21. Would you be open to using a new, efficient and fast sampling method to determine mycotoxin contamination that would reduce the costs and efficiency of traditional sampling methods and has a built-in detector (MYCOHUNT)?

- ☐ Yes and would be happy to receive more information
- ☐ No, I would not be able to make use of such a product
- ☐ I do not know but would be happy to receive more information

22. How much would you be willing to pay for a hand device that measures mycotoxin contamination?

- ☐ I cannot afford to buy any device that is more expensive than 2000€
- ☐ Between 2000€ and 5000€
- ☐ More than 5000€
- ☐ I do not know

23. How much would you be willing to pay for an automatic and efficient sampling and measuring system?

- ☐ I cannot afford to buy any device that is more expensive than 5000€
- ☐ Between 5000€ and 10000€
- ☐ Between 10000€ and 20000€
- ☐ More than 20000€
- ☐ I do not know

24. What is the most important feature of a grain sampling and measuring device for you? (rank the importance, most important is 1, the less important is 4)

- ☐ Performance/capacity
- ☐ Speed
- ☐ Reliability
- ☐ Price

25. Is there any subsidy available for you to purchase an innovative sampling and measuring device?

- ☐ Yes
- ☐ No
- ☐ If yes, please specify _____

27. Would you like to be informed of the project results of Mycohunt?

- ☐ Yes
- ☐ No

26. Personal details (Optional)

Contact details (optional)

Company name:

Contact person:

Email address (necessary if you need update on the progress of the project):

We will email you only the requested information. The information collected from the questionnaire will be used only for project purposes and no third party will get access to your contact details. The questionnaires will be processed anonymously.