



International Association for Soaps,
Detergents and Maintenance Products

Enzyme Safety Management

A series of web based training and Information
Sessions developed and presented
by the AISE Enzyme Safety Task Force



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Session 4: Exposure monitoring

Christiaan De Vos,
Sustainability manager, EMEA
DuPont, Industrial Biosciences



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Good afternoon everybody; thanks for being with us today. I'm Chris De Vos, EMEA sustainability manager for DuPont, Industrial Biosciences and located at the Brugge site in Belgium.

I've worked already 32 years for the company; I'm actually conducting also a lot of air monitoring at both our own production sites and at customer facilities to cover all identified uses in view of the update of our Reach submissions.



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Objectives of this webinar

Acquiring understanding what the benefits are of air monitoring :

→ Allows us to quantify employee exposures

- Evaluation of risks
- Degree of Hazard

→ Helps us to evaluate the control measures

- Effectiveness of engineering and work practice controls
- Need for personal protective equipment



→ Ultimate goal : Preventing Employees to get sensitized and/or converting to Allergy

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What are the objectives of this webinar ? I think the objectives should be clear; we need to understand where the engineering controls are inadequate to control aerosol concentrations to an acceptable level; at those places or activities, people should wear a P3 respirator. It's clear that our goal here is : "zero sensitizations" . In one of the next slides, I'm gonna talk also about the challenges of the 'air monitoring program'



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Air monitoring : when ?

- in conjunction with the semi-quantitative tools of performance assessment of equipment & behavior
- also in conjunction with the outcome of the medical surveillance
- presence of visible dust/liquid aerosol :
exposure probably above the OEL (Occupational Exposure Limit), hence 'no need' for air monitoring.



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When to conduct air monitoring ? If we look at this slide, generally spoken, enzyme air monitoring should be prioritized based on the risk of exposure to workers,..that is key. First we can start with a qualitative assessment of the risks, as this will be the input to design the site's air monitoring strategy. It's clear that the strategy will be depending also on the outcome of medical surveillance – for instance – when immunological testing reveals a changing trend in incidence of sensitization. And definitely, the strategy will also be depending on the outcome of the performance assessment of the equipment, the work practices and behavioral aspects.



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Components of an air monitoring programme

- Training
- Air sampling equipment
- Air sampling Plan/Strategy
- Data analysis and interpretation
- Feedback to employees
- Corrective actions and follow-up



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These are the most relevant components of a sound air monitoring program; I'm coming back on this later, but don't forget to involve your employees on the work floor, as they know best what's going on. If they are part of the monitoring team, it will help afterwards to communicate the results to the employees, especially when it comes to defending unpopular measures, such as the mandatory imposition of the use of respirators



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Training : objectives

- How to establish a sampling plan
 - frequency, location, time
- Selection of monitoring equipment
- Able to evaluate data
- Assessment of the adequacy of control measures/advice for corrective actions
- Operation/calibration of sampling equipment
- Hygienic practices

→ remember : the slightest contamination will ruin a sample !!



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Training of the monitoring team is very important; I have to say that even after doing this job for 32 years, I'm still learning every day. The biggest risk here is to contaminate a sample. Please recall that the objective is to measure nanograms of enzyme (billionths of a gram). High flow sampling filters are especially susceptible to contamination because the filters are not housed in a cassette and have a large surface area exposed to the environment, this in contrast to the 37mm filter cassettes. Tweezers and high-flow filter housing should be cleaned with ethanol between uses. Other important aspect of the training is the sampler calibration, but we'll come back on this later in more detail.

Air sampling equipment



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- High volume samplers : intended use

→ where you need to collect large volumes of air in a short period of time, also because some analytical methods may have high LOD's. (limit of detection), which may compromise the use of low volume samplers

- Low volume samplers : intended use/limitations

→ personal sampling/area sampling
→ more sensitive analytical methods may be needed

Example: 37 mm Glass Fiber Filter Cassettes or GH polypropylene (**filter material depending on enzyme to be monitored**)



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As to the equipment....The choice between High or Low volume sampling depends on the purpose of the monitoring. If the purpose is to establish baseline airborne enzyme concentrations in an area, and the process conditions are stable for a couple of hours, we can easily make use of low volume samplers and let them run for 2 hours in the neighborhood of the operator's breathing zone. Adversily, if the process varies in a manner that could create peak exposures over a short period of time (for instance filling a process vessel with a liquid enzyme discharges enzyme aerosols from the tank vent) , the use of a high volume pump might be more appropriate.



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High Volume Air Sampling

- 300 liters/min for liquids/aerosols (lower rate to avoid loss of aerosols), up to 600 liters/min for solid particles
- 15' for peak exposures (always be “averaged out” by sampling systems that accumulate a sample over time)
- up to 4hrs for area sampling
- Samples at a fixed height (operator breathing zone, 1.5 m)
- Samples omnidirectional (360°)
- Sample head design simulates the human breathing velocity (1.25 m/s)



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So, these are a couple of bullet points indicating the particularities of high volume sampling; again, most important difference with the low volume samplers is the ability to identify peak concentrations which could lead to unacceptable inhalation exposures for the workers involved in that specific activity.



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High Volume Samplers: examples of commonly used devices

Suppliers:

→ Gravikon, Bendix Sensidyne, Graseby, Tisch, Cerulean,....

→ original Galley sampler :

Designed by John Galley [Lever Engineering] in about 1970 & recommended by SDIA in 1973. Formerly distributed by Newton Instruments, now Cerulean



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The height of the filter is set at the average height of an operator (just over 1.5 meters) and the filter head is precisely manufactured so that the speed of air flowing into the filter is similar to the speed of air breathed in. The filter is also shielded from excess contamination by a hinged plate.

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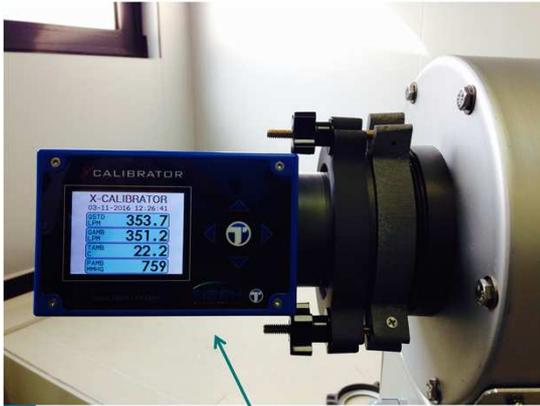
**Example:
Tisch Environmental sampler**



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Industrial Enzyme Sampler



Easy calibration



“Portable” and can be mounted on a tripod

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Giving you an example of the newly developed and easy-to-use X-calibrator from Tisch.

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Gravikon VC 25 II (New design)



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Dust collection device Gravikon VC 25 II



Our new dust collection device Gravikon VC 25 II is designed for stationary sampling of dust at work places with a controlled flow rate of 22.5 m³/h. The sampler combines new technologies in operation and flow control with proven and recognized dust collection.

With the original interchangeable sampling heads of the former VC 25, type Ströhlein, the collection of the inhalable and respirable dust fraction acc. to EN 481 is guaranteed. The filter cassettes for filter diam. 150 mm enable easy handling and safe transport and shipment to the lab for subsequent gravimetric and analytical evaluation.

Dimensions : (LxWxH) 21 x 25 x 48 cm ,
Dimensions incl. Stand (LxWxH) : 21 x 25 x 159 cm
Weight: 6,5 kg

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This one is widely used in Germany; please note the very high flow rate

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Low Volume Air Sampling

- Mode of operation
 - 2-3 liters/min
 - personal or area sampling
 - easier to calibrate
 - no external electrical source needed
- Suppliers
 - Sensidyne (Gillian, AirCon), SKC, Casella...



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The big advantage here is the portability of these pumps, also because they do not require an external electrical source for operation.

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Examples of low volume samplers



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Example: Aircon 2 sampler (Sensidyne)

- 25 L/min (still considered as low volume sampling)
- ideal for targeted exposure measurement (peak exposures)
- You can direct the sampling head to the equipment/operation that you want to assess



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This device, running at 25 l/min, enables us to go for targeted exposure measurement or peak exposures, keeping the sampling times quite limited and with the advantage of the portability, allowing sampling at otherwise difficult accessible places.

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Air Sampling equipment

Types of Calibration Standards

→ Primary Standards - make direct measurements of the volume of the device.

Examples: soap-bubble meters, spirometer, Gilibrator, Buck Calibrator, Defender



→ Secondary Standards - standard calibrated against a primary standard (must be calibrated with 1° standard to be accurate and precise)

Example: precision rotameter



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As said before, calibration of the equipment is crucial for the reliability of the results; pumps must be calibrated before and after sampling with the sampling media in place to verify that the sampling rate remained constant. It's a good practice to discard samples if the flow rate changed during the sampling by more than 5% of the initial flow rate

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Primary standard: examples

Example: Defender 530

Accurate, Reliable & Portable
Primary Calibrator
for Fast Calibration of Air
Sampling Pumps



Example : Gilibrator



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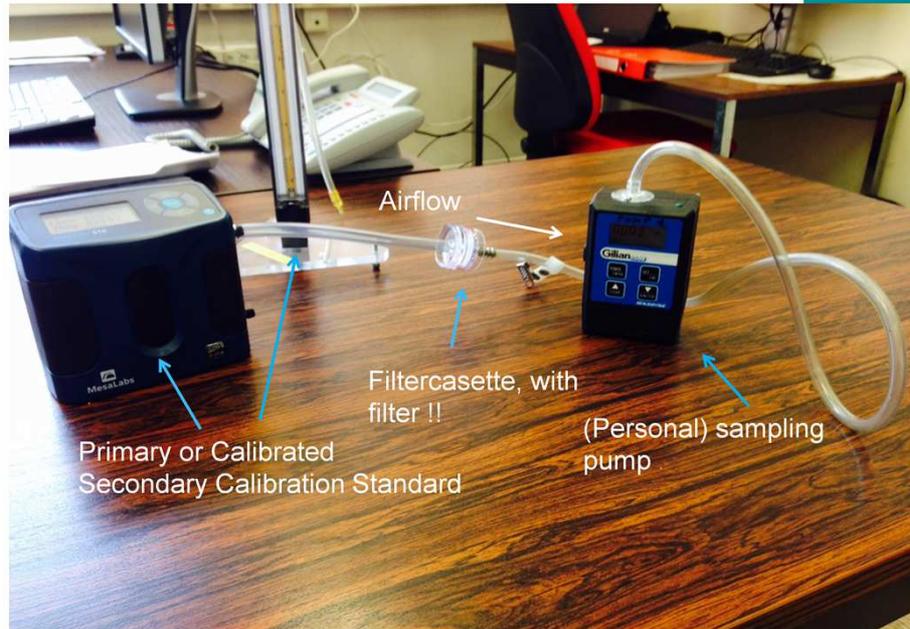
Some examples of primary standards; don't forget though to get them re-calibrated by your supplier on annual basis, and ask for the certificate. This might be helpful in case authorities would come in and review your occupational exposure assessment program.

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Calibration set-up

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Here you see a picture of what the calibration and sampling train configuration could look like. For the sampling itself, the intake of the high volume pump (e.g. important for the Tisch sampler) should be oriented towards the most likely source of enzyme exposure. Filtercassettes should be angled downwards towards the floor during low-flow sampling to prevent contamination from large particulates.

Air sampling plan



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1. Familiarize yourself with the process
2. Set objectives
3. Note engineering controls and housekeeping
4. Observe work practices: link with visual assessment !
5. Observe environmental conditions/potential sources of release
6. Understand which products are being used

This **qualitative risk assessment** should be utilized to design the site's air monitoring strategy.

Based on the preliminary data, determine sample size, types of samples to collect, when and where to sample, and sample duration



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What about the air sampling plan ? We already highlighted this before but again, in order to really understand what's going on in the plant, involvement of workers in the field in the execution of the air monitoring program is crucial. It would not be the first time that enzyme concentrations may differ drastically from results in the past, because of changing environmental conditions or changes to any part of a local exhaust ventilation system. I have seen cases where due to the installation of a biosafety cabinet, underpressure was created in an otherwise clean area, leading to intake of contaminated air from an adjacent production area, because supply of make up air wasn't anticipated in the project, a typical example of a failing "management of change". It's not abnormal that an occupational hygienist, not necessarily involved in the daily plant operations, could have overlooked this, and again, that's why we definitely need people in the field.

Air sampling plan

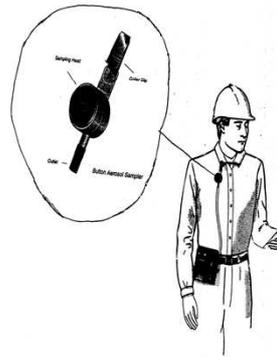


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Where to sample ?

1. **Point Source** - Point of operation or generation
2. **Area** - Near the workstation or at the border of the work area.
3. **Personal breathing zone (PBZ)**

- < 30 cm sphere around head, 1.5 m high
- not the preferred method though !!
(peak exposures may be averaged out)
- may be required from authorities
(requirement environmental permit ?)
- Ref: - EN 689:1995 and EN482
 - Council Directive 98/24/EC
(07/04/1998)
 - ECHA Guidance, Part R.14 :
"Occupational Exposure
Assessment"



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Just this slide to give you an overview of the 3 sampling approaches that may be used; although you might be pushed by authorities or guided by EN standards to opt for personal monitoring, we believe that "personal breathing zone" sampling is not the preferred method for enzyme exposure monitoring, for a couple of reasons; first of all, personal samples will not necessarily identify peak concentrations, adversely to high volume area or static sampling and point source sampling. Secondly, operators nowadays are working more and more from out control rooms, are multitasking, hence not necessarily staying for the full time at the defined sampling location. Finally, personal monitoring doesn't provide a useful way to ensure engineering controls such as containment and LEV are working effectively.



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Air sampling plan

Sampling locations : general guideline

- discharge of enzyme supply container
- enzyme dosing area at dosing units
- tableting area
- mixing or blending of finished product
- powder/liquid storage area
- finished product storage/transfer areas
- head of packing machine
- packing reject station
- in area set aside for recovery of powder



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Besides the locations highlighted here, which are typical for a detergent plant, it's also of utmost importance to focus on non-routine activities, such as trouble shooting, rework activities, handling of enzyme (packaging) waste, ...hence, all activities which may create peak exposures, this in contrast with normal routine activities, where generally exposures are well controlled.



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Data analysis and interpretation

→ Performance indicators : **CpK** (Capability Ratio) and **UCL** (Upper Control limit) , see also AISE's guidance document

$$\text{CpK} = \frac{\text{OEG Value} - \text{Average Value}}{3 \times \text{Standard Deviation}}$$

CpK is a measure of how capable a system is of delivering results below the OEG: the greater the CpK, the higher the capability.



As to the UCL, this indicator is typically set at 50% of the OEG (or DMEL)

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Data interpretation is perhaps the most difficult part of the whole exposure assessment, because of :

- First, do you want to use this data for the decision making whether or not capital investment is needed to improve engineering controls ?
- Secondly, do you want to use this data to motivate people wearing the needed respiratory protective equipment.

Anyway, the data from each specific location or activity should be analyzed separately as its own dataset.

How to interpret this formula of the CpK in another way ?....if the average of 3 or more measurements plus 3 standard deviations provides a value less than 60 - this means also that the CpK is > 1 - than the dataset can be considered valid and suggests that respiratory protection is not required in that area or for that task.



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Data analysis and interpretation

→ **Important to give feedback to employees**

Results are above OEG (DMEL) : employees informed and wear RPE !!

Advice : have an air monitoring team with production operators :

→ **increases involvement**

→ **enhances communication**



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As referred to in former slides, I cannot enough emphasize to get your operators involved in this program, which is especially needed to obtain buy-in from the whole workforce when it comes to taking un-popular measures, like the mandatory use of respirators.



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Interpretation of results according to REACH

Part R.14 : Occupational Exposure Assessment

- Basis is the **RCR = Risk Characterisation Ratio**, which is the quotient between the exposure level and the DMEL
- The closer the RCR is to 1, and the higher the variability of the data (high SD), a higher number of data points is needed
 - from a min. to 6 up to 25 samples per location/activity



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Although not in our AISE's guidance document, we could not make it to not mention part R.14 of ECHA's Guidance on Information requirements and Chemical Safety Assessment, in particular : "the Occupational Exposure Assessment". The former version of the R.14 required even more samples per location in case of high RCR's and high variability and uncertainty of the data.



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Corrective actions and follow-up

Sampling Results out of the OEG limit :

- take immediately remediation steps
- may involve temporarily stopping production

Follow-up needed to ensure that corrective action was effective



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I think these corrective actions are self-explaining; again, a good follow-up will consider also the “management of change”.



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What are the challenges of 'exposure monitoring' ?

- No 'real-time' monitoring
- Are we able to adequately monitor "peak exposures" ?
- When comparing monitoring results with the DMEL, are synergetic effects taken into account ?
- Rate of uptake of hazardous substances varies with the different degree of physical exertion.
- EN689 allows "health expert advice" based on only 1 datapoint if the result is < than 10% of the exposure limit.
- Just because of the definition of a DMEL, monitoring results below the DMEL do not guarantee a zero sensitization incidence.
- Denaturated enzyme (high T°, low pH,.....) with a largely intact protein structure may not respond to the current analytical assays but may still elicit an immunological response.



→ hence, focussing on "engineering controls" is crucial !!

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At the beginning of this webinar, I informed you I would come back on some challenges....So far, the development of real-time analyzers has been a challenge; working prototypes have been constructed, although they didn't really pass the commercialisation phase. With the actual tools and analytical methods, we are always behind the facts. Therefore it might be difficult to trace back what went really wrong at the time of the sampling. Other challenges are reflected by the other bullet points and are quite obvious.



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Analytical procedures

2 methods available – check with your enzyme supplier for more accurate data regarding the LOD's (limit of detection) !

- **Enzyme activity-based assays**
L.O.D. ~ 1-1.5 ng enz.protein/ml

- **Immunoassays, like ELISA**
(Enzyme Linked Immuno Sorbent Assay)
L.O.D. ~ 0.1 ng enz.protein/ml



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One aspect of the exposure monitoring is the sampling, but the analytical part is even as important. We are referring to these common two methods, of which the activity based assays will be still the most practical ones, especially for the small and medium enterprises. Please ask your enzyme supplier for the customer analytical methods.



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Analytical procedures

Enzyme activity-based assays :

- Easy to set up and run
- Activity measured photometrical
- Based on commercially available substrates
- L.O.D. sometimes borderline
- Assay principle and instrumentation should be considered for every new enzyme
- Manual or automated (auto-analyzer)



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As to the activity based assays....they are easy to set up and run, but the higher LOD's may be the limiting factor. Important for the validity of the air monitoring results is the analysis of the so-called 'blanks'. Blanks are analyzed to rule out sources of contamination and analytical error. A buffer blank should be collected on each day that the buffer is used. At least one filter blank should be analyzed to rule out contamination of the filter stock.

In addition, air monitoring assays require prompt analysis. Enzyme activity decreases over time so filters should be preserved in a buffer as soon as practical after the sampling is performed. Filter cassettes should be refrigerated if the filters cannot be placed in a buffer on the day of the sample collection. Samples can generally be kept in buffer for a few weeks when stored refrigerated. This info should be available in the validated SOP's for the air monitoring assay developed by your enzyme suppliers.



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Analytical procedures

Enzyme activity-based assays

- Based on the chemical reaction of the enzyme [e.g. protease] with e.g. N-succinyl-ala-ala-pro-phe-p-nitroanilide
- Cleavage of p-nitroanilide from the substrate results in an increase in absorbance at 410nm
- Intensity of colour is proportional to the enzyme concentration provided that all the conditions are standardised.
- Suitable for auto-analyser techniques.



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This is just an example; other substrates and reagents may be used for other enzyme classes. There is a wide variety of auto-analysers,....Cobas, Konelab, Skalar,...to name a couple.

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Analytical procedures

Immunoassays (ELISA)

- High specificity
- Low L.O.D.(limit of detection)
- Measures immunochemical activity
- Same principle and instrumentation used for all methods
- Demands trained staff and clean room
- Demands specific antibodies
- Time consuming and costly (development antibodies)



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Elisa assays rely on binding of the enzyme to specific antibodies in order to quantify protein. They also provide a more toxicologically relevant measurement. Although Elisa techniques are able to go for very low detection limits, it requires additional equipment and specialized training, so less obvious for normal 'production quality control labs'.



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Dustiness of enzyme raw materials

- Quality of the raw material may be a prediction tool for 'performance' in the plant
- Equipment shear forces may result in high dust levels where poor quality enzyme granular material is used !
- Quality control : 2 methods
 - Vertical Elutriation Test
 - Heubach Attrition test



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Let's also spend a couple of minutes to the characteristics of the enzyme raw material, here in particular : the dustiness, as the quality of the enzyme encapsulate may be a predictive tool for initial evaluation of the exposure risk at the plant. 2 methods are available, quite different in their objective.



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Vertical Elutriation Test

- **Objective** : quantify amount of free dust of an enzyme encapsulate
60g of encapsulate placed in an airflow at 0.8 m/sec for 40' → small particles are collected by filtration and than analyzed for dust (gravimetric) and enzyme (activity or Elisa)

→ **Assures the quality of the encapsulate regarding 'free' enzymatic dust**



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Elutriation equipment



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Here you see a picture of an elutriation equipment.



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Heubach Attrition Test

Objective : ascertain the strenght/durability of an enzyme encapsulate by subjecting them to a physical force (steel balls) → measuring dust as a result of the attrition

→Predicts how encapsulates may be affected by a given process



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Adversily to the vertical elutriation test, this test predicts how encapsulates may be affected by a given process

Example: Heubach equipment



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And here is a picture of a Heubach instrument; please be aware that cleaning of the Heubach instrument parts may create aerosols (we have measured levels up to 143 ng/m³ during sample preparation and cylinder cleanout) , so please take your precautions ! Compressed air cleaning is a documented risk factor for enzyme exposure !

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Filter Analysing



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- Airborne enzyme concentration =
ng protein/sampling rate (m³/min) x time (min)
- Method sensitivity : sufficient to detect 10% of OEG for each enzyme type
- Enzyme recovery efficiency :
 - for dust samples : ~ 100%
 - for aerosols : ~ 50-100%,
depending on enzyme and filter type used (ask enzyme supplier !)
- Adopt good hygienic practices to prevent contamination of buffer and filter pads



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Be aware that each enzyme has a unique affinity for the filter material. Evaluating the amount of protein that can be recovered from the filter and defining the elution conditions that optimize the recovery are important steps in developing a valid assay. A recovery factor is assigned to each enzyme as part of the assay development and validation process. Be also aware that these recovery factors may be different for dust and aerosols. So, please ask your enzyme supplier for these recovery factors.



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Filter Analysing

- Recovery efficiency of aerosols and dust from filter media in different sample matrices should be validated.
- Done by enzyme spiking experiments :
 - Enzyme recovery efficiency with background matrix present
 - Check background matrix response to a given substrate
 - Stability of enzyme in background matrices



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So again, this is what we just came to tell. In addition, for the detergent plants, response of the detergent matrix to a given substrate should also be evaluated. That's why it's important to run some 'field blanks', i.e. detergent matrix without the enzyme.



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Case study 1

Don't overlook activities in your laboratories!

High enzyme concentrations have been measured during seemingly benign activities like rinsing glassware and stirring concentrate



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I wanted to share with you also 2 case studies. The first one : don't overlook activities in your laboratories !



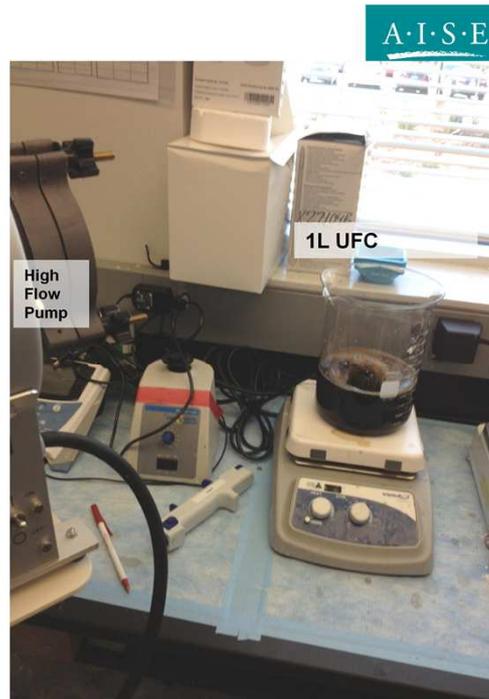
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UFC stirring (open)

Stirring 1L protease UFC at full vortex = 465 ng/m^3



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So, what would be the precautionary measure here ?



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UFC stirring (covered)

Stirring UFC at full vortex COVERED = $<16.6 \text{ ng/m}^3$



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Right, you got it...just cover the beaker



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LEV

Case study 2

Thinking back at what we learned
during former webinars.....

What's the bad practice here ?
(lab technician is pouring samples
in the sink)



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The second case study....thinking back t what we've learned in an earlier webinar....a lab technician is pouring enzyme samples in the sink.....What is the bad practice here ?
.....Look at the position of the LEV.
Right, you got it !

What is next.....Future Webinars



→ Past webinars :"

- Introduction to "enzyme safety".
- Engineering Controls
- Risk management measures

This webinar from the AISE Enzyme Safety Task Force has introduced you to the principles and techniques of "exposure monitoring".

Future webinars will focus in more detail on:

- Health Surveillance – For Site Management, Safety Managers, Occupational Health
- Performance Monitoring – For Site Management, Safety Managers, Occupational Health
- In addition to the webinars, training material (presentations) will be published on the topics below:
 - Consumer Safety – For Product Development, Product Safety, R&D (March 2016)
 - Laboratory Safety – For Safety, Laboratory & Quality Managers, Laboratory staff (April 2016)



Any Questions ?





•On Behalf of the AISE Enzyme Safety Task Force

Thank You For Attending Today

**We Will Appreciate Your Feedback or Further
Questions to:**

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