

Final Documentation

Team Schrodinger – Unicorn DX

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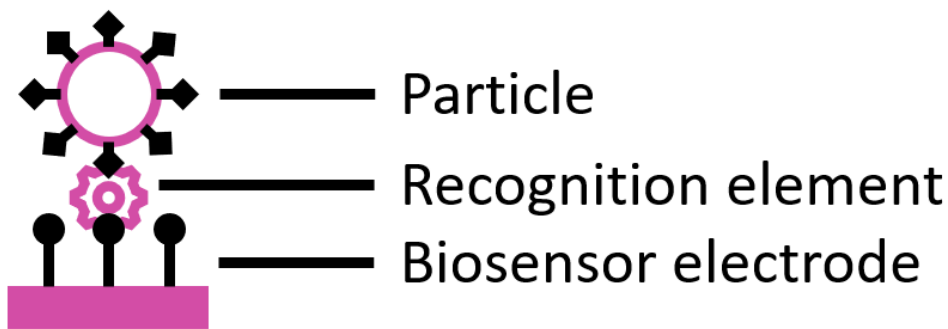
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1. Unicorn DX technology



Unicorn DX is an electrochemical biosensor that detects predetermined particles in liquid using recognition elements¹; see the figure above. A recognition element can be, for instance, an antibody. Antibodies are more or less specific; some bind to families of particles, while others bind to specific particles.²³ The technology also allows for the use of a thousand recognition elements simultaneously, and there is no apparent limit to its capacity.⁴ Hence, it could probably be used to, e.g., detect BAB-Spores in general, or a specific BAB-Spore.

The detection process involves putting voltage through an electrode that generate a current through the detected particle and back to the sensor. The presence of a current indicates the detection of a particle. This is more or less general for biosensors⁵. Setting Unicorn DX apart, it uses a combination of cutting-edge biosensor techniques. For instance, the surface coating makes it less sensitive to environmental factors and quicker than its predecessors. Additionally, it utilizes nanotechnology to detect particles ranging from 10 to 100 nanometers, while other modern biosensors with surface coatings detect microparticles.⁶

Possibilities

Unicorn DX can be combined with other similar technologies, such as flow cytometry as the techniques are similar and could use the same micro channel.⁷

It is possible, with (a lot of) re-engineering, for the technology to work continuously. For instance, it would be possible to engineer a feature where the whole coating is striped and fresh one was applied. This would require a lot more valves, reservoirs, channels and elegant flow control, but it is not impossible.⁸

¹ <https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2020178408&tab=PCTBIBLIO>

² <https://www.youtube.com/watch?v=7i4e6ycymkE>

³ Written communication with Occam DX 2023-04-14

⁴ Interview with Occam DX, 2023-04-18

⁵ <https://www.youtube.com/watch?v=gXw7armpsEw&t=111s>

⁶ Interview with Occam DX, 2023-04-18

⁷ Interview with Occam DX, 2023-04-18

⁸ Written communication with Occam DX 2023-04-14

Limitations

Interview with Occam DX, 2023-04-18:

The current set-up requires a bit of training before for the operator to understand what's going on, as they need to understand the electrochemical results. If a computer does the interpretation the output could be a simple number – BUT the simpler the tool, the longer it takes to develop and the more expensive it will be.

The sample size is currently 1 ml. It could be increased but the electrodes are on the surface of the chip. So, if the sample is too large, it won't be possible to detect sensible particles. Larger samples also take more time. However, it is possible to do parallel process, e.g., one could use 15 channels. If a big sieve is engineered, sensors could be put in each corner. If there are a million spores, then a million detectors are needed. Hence, the effectiveness of Unicorn DX in detecting small concentrations such as one bacteria per liter is not satisfactory.

Written communication with Occam DX 2023-04-14:

Occam DX uses disposable one-time-use cartridges, because of both regulative limitations (danger of cross-contamination) and technical limitations of the product itself. The chip would probably still work a second or third time if a good cleaning protocol was developed, but it not certain. For sure, both the technological development as well as the certification process of a reusable system would be complex.

2. Process of design



In the last 5 weeks this group has performed research into potential interesting markets for the Unicorn DX technology. While currently focused on particle detection in the medical field, the organisation has their eyes open for other industries. In this 5-week process multiple ideas have been generated through a variety of methods including brainstorm sessions, story games and individual inspiration. Most of these ideas have been researched at A surface level, but the further we filtrated possible ideas, the more we increased depth. In the end one idea has been put forward as most suitable, as result of the 5-week long process.

During the first week it was established that anything to do with the medical field, bodily fluids or animal diseases would not be counted towards finding a new market for the current technology. These ideas have been scrapped. In week 2 the group held brainstorm sessions and created numerous interesting industries at surface level. Including irrigation, nano fertilizers and nano pesticides, wastewater, water sanitation in refugee camps, rare metals, paint, toothpaste, detergent, glass cleaner, asbestos, sugar cane juice and hotel & restaurant. Towards the midway point these options have been dropped due to lack of feasibility, difficulty, or competition. The list has been reduced to 6 options, recreational water, drinking water, fruit juice, batteries, wine and milk.

After the final meeting with the Unicorn DX organisation the selection has been made to focus on wine and milk production, based on the received feedback. The focus was on detecting fungi in wine or BAB-Spores in milk with use of Unicorn DX technology, with the latter finally having the preference of the research group.

3. Most promising idea: Testing for bacterial spores in dairy milk

What are we looking for in milk?

There are various components that can cause damage in dairy production or be harmful to human consumption in milk. Examples would be:

- Salmonella
- Listerine
- E.Coli
- Pseudomonas
- Bacillus cereus
- Clostridium
- Campylobacter⁹

3.1 Information on BAB-spores

Clostridium tyrobutyricum can be said to be the main spoiling agent for semi-hard and hard cheeses, however, there are also other clostridial species such as Clostridium butyricum, Clostridium sporogenes or Clostridium beijerinckii, that have been set into connection with late blowing cheese defects.¹⁰

Taking the example of Clostridium tyrobutyricum, it is a gram-positive anaerobic rod with a size ranging between $1.9\text{--}13.3 \times 1.1\text{--}1.6 \mu\text{m}$.¹¹

The spores of these bacteria are often seen as the main cause for the so-called late-blowing defect in semi-hard and hard cheeses. The spores produce butyric acid, acetic acid, hydrogen, and carbon dioxide, which during the maturation of cheese causes a burst of the cheese load as well as an undesirable taste, ultimately making the cheese unsuitable for sales, causing economic losses for the producers. The spores are able to survive heat treatment, such as pasteurization. Bacteria can enter the raw milk through infected teats, milking cups, bulk tanks, bedding, excrement, feed, or milking equipment. Furthermore, contamination can happen during transportation from the farm to the processing plant as well as inside the processing facility due to careless handling and infected equipment.^{12,13,14,15,16}

⁹ [Mikroorganismen in Milch: Die 7 größten Bedrohungen bei der Produktion \(r-biopharm.com\)](#)

¹⁰ [Biosensors | Free Full-Text | Colorimetric Point-of-Care Detection of Clostridium tyrobutyricum Spores in Milk Samples \(mdpi.com\)](#)

¹¹ [Clostridium tyrobutyricum - an overview | ScienceDirect Topics](#)

¹² [Detection of butyric spores by different approaches in raw milks from cow, ewe and goat - ScienceDirect](#)

¹³ [Biosensors | Free Full-Text | Colorimetric Point-of-Care Detection of Clostridium tyrobutyricum Spores in Milk Samples \(mdpi.com\)](#)

¹⁴ [Validation of a real-time PCR method on pta gene for Clostridium tyrobutyricum quantification in milk - ScienceDirect](#)

¹⁵ [Development of a new multiplex quantitative real-time polymerase chain reaction method for Clostridium butyricum, Clostridium sporogenes and Clostridium tyrobutyricum detection in cheese - ScienceDirect](#)

¹⁶ [Frontiers | Detection and Enumeration of Spore-Forming Bacteria in Powdered Dairy Products \(frontiersin.org\)](#)

Impact of BAB-spores in aged cheese & milk production

For grade A milk producer, the US Pasteurized Milk Ordinance's regulatory limits for public health protection are 750,000/mL bulk tank SCC and 100,000 cfu/mL SPC for example. The majority of farmers work to uphold stricter standards that are frequently correlated with quality rewards or "premium" payments provided by cooperatives or other raw milk buyers. The premium payments for these incentives are frequently tier-based, with higher-quality raw milk receiving a greater reward. Combinations of tier targets for SCC and bacterium counts typically range from 100,000 to 350,000 cells/mL and 5,000 to 20,000 cfu/mL, respectively. Although the goal of providing financial incentives for higher-quality raw milk may be to simply encourage and reward dairy farmers for their efforts, processors are more likely to do so in order to get a return on their investment by paying for high-quality raw milk that enables more effective processing and the production of higher-quality products.¹⁷

For a raw milk screening test, a sensitivity of two spores per 10 ml is required since as little as one or two *C. tyrobutyricum* spores per 10 ml of milk can result in the late-blowing fault in cheese.²¹

Current testing methods

In order to consistently produce high-quality dairy products, processors are requiring higher-quality raw milk, which can be characterized as (1) compositionally complete (for example, protein and fat levels within the norm), (2) free from off-flavors and odors, (3) free from detectable drug residues, added water, or other adulterants, (4) having low total bacterial counts, and (5) having low SCC. When supplies arrive at the dairy processing facility and at the producer level, processors frequently check them to make sure they are utilizing high-quality raw milk.¹⁷

For the purpose of determining the overall concentration of bacteria and antibiotics in the milk, representative random samples of untreated milk from randomly chosen milk production facilities should be analyzed. The process is laid down in EC Regulation No. 853/200454. The following criteria must be met for the total quantity of bacteria in the milk, which should be tested twice a month.¹⁸

More focused microbiological testing is becoming increasingly necessary, for example, for endospore (spore)-forming bacterial groups that could endure processing and result in further flaws in some goods.¹⁷

Strict restrictions are in place regarding the permitted quantity and species of microorganisms present in milk to meet quality and safety standards in the dairy industry. Spore-forming bacteria must get special attention because of their innate capacity to endure harsh processing conditions. In more detail, the multilayer-structured dormant endospores produced by some species of bacteria, known as spore-forming bacteria, which can occasionally be pathogenic, can withstand extreme environmental stresses like desiccation, high pressure, high and low

¹⁷ [Influence of raw milk quality on processed dairy products: How do raw milk quality test results relate to product quality and yield? - ScienceDirect](#)

¹⁸ [FULLTEXT01.pdf \(diva-portal.org\)](#)

temperatures, UV radiation, and chemical stress. Constraints of conventional microbiological detection techniques are related to time, efficiency, precision, and sensitivity.^{19,20}

Most Probable Number

The most popular method for determining the amount of *C. tyrobutyricum* bacteria in milk is now the MPN process, which uses three or five tubes.²¹

The Most Probable Number (MPN), a semi-quantitative microbiological approach that calls for fermenting a liquid medium containing lactate as an organic molecule, as the conventional reference method. Using this technique, gas generation under anaerobic circumstances may be detected after vegetative cells have been destroyed and spore germination has been induced by heat treatment. However, MPN takes a lot of time since it needs a seven-day incubation period to produce gas and display broth turbidity in positive tubes, which is incongruous with the timetable of cheese manufacturing. Due to these extensive pre-testing steps the entire testing process can take 3-10 days and requires a laboratory setting. Additionally, MPN yields ambiguous findings because not all spores germinate in the culture medium and because facultative anaerobic spore-formers may obstruct the identification of clostridia as a result of poor media selectivity. Additionally, this approach is unable to differentiate between various spore-forming bacteria. Notwithstanding its disadvantages, the benefits of this method are its possible large sample analysis and low cost implications.^{24,22,24}

The detection threshold for the MPN method appears to be 18 MPN/L. Assuming a mean concentration of 69MPN/liter (1.79 log₁₀ MPN/liter), spore levels appear to vary greatly per farm.²³

PCR-Testing

A slightly faster testing method delivering results within 48h has also already been introduced, which however still does not provide the benefit of testing speed. In recent years, research has been performed on real-time PCR testing for spore-forming bacteria within milk with the aim to develop a testing method that provides more specificity, accuracy and speed compared to the earlier mentioned methods. Nevertheless, pre-testing steps are still necessary such as DNA extraction coming with several incubation steps which again prolong processing time and require the usage of a laboratory. Moreover, PCR-based techniques pose a significant cost as well as skill burden on the cheese producer and are consequently not suitable for a daily application.^{22,24}

Other investigated methods – Direct Microscopic Somatic Cell Count Method

Milk or certain milk products can be checked for the presence of bacterial clumps or somatic cells using the direct microscopy approach. Bacterial clumps may be counted in the first

¹⁹ [Occurrence and identification of spore-forming bacteria in skim-milk powders - ScienceDirect](#)

²⁰ [Frontiers | Detection and Enumeration of Spore-Forming Bacteria in Powdered Dairy Products \(frontiersin.org\)](#)

²¹ [Most Probable Number - an overview | ScienceDirect Topics](#)

²² [Validation of a real-time PCR method on pta gene for Clostridium tyrobutyricum quantification in milk - ScienceDirect](#)

²³ [Butyric acid-producing bacterial spore levels in conventional raw milk vary by farm - PMC \(nih.gov\)](#)

²⁴ [Detection of butyric spores by different approaches in raw milks from cow, ewe and goat - ScienceDirect](#)

application, the direct microscopic clump (DMC) count, while also being evaluated for their unique shape and organization. Cell morphology and clump arrangement only partially assist the analyst in determining the root cause(s) of quality issues. Large somatic cell counts in the latter case suggest mastitis or other aberrant udder diseases. The microscopic approach has limited but potentially useful applications for figuring out how much fluid pasteurized milk and cream is contaminated with germs. This approach is not utilized to check for finished product compliance because dying cells lose part of their stain-absorbent capacity and because pasteurized milk products often have low bacterial counts. However, this approach does have utility as a quality control tool because, regardless of the type of organisms present or whether they are viable (alive) cells, huge quantities of bacteria in uncultured items offer signs of filthy circumstances.²⁵

Other investigated methods – Preliminary Incubation Count Method

A reliable microbiological test is the preliminary incubation (PI-SPC) count, which can reveal the hygienic status of the load and/or specific manufacturers. It highlights the presence of psychrotrophic bacteria, which may grow very quickly at refrigeration temperatures. In order to comply with legal requirements and quality standards, it is utilized to regulate and monitor the temperature throughout the facility.²⁶

How can Unicorn DX help?

There have already been brief empiric studies that state that in addition to their selectivity for the target analytes and high accuracy in such complex matrices, biosensors represent an innovative, quick, and on-site alternative to evaluate milk quality. The majority of biosensors use enzymes, aptamers, or antibodies as the bio-receptor and generate a signal through optical, electrochemical, or thermometric transduction. Although they have more complicated designs, electrochemical sensors are more precise and sensitive and a very interesting future possibility for the dairy industry.²⁷

How can BAB-spores be avoided entirely during cheese production?

By treating raw milk with bactofugation, microfiltration or bacteria additives the late blowing defect in cheeses can be reduced, however, all of these methods also come with their own technical or regulatory limitations.²²

A bactofuge is commonly used in the early milk processing steps for cheese production and is able to remove spores and bacteria or concentrations of proteins with an effectiveness of 99% or more, given that two or more bactofuges are installed in a series in the cheese milk processing procedure.²⁸

Contaminated milk can without any health risk be used for other dairy products such as regular milk or the production of non-aged cheeses.²²

²⁵ [Chapter 10 Direct Microscopic Methods for Bacteria or Somatic Cells | Standard Methods for the Examination of Dairy Products \(aphapublications.org\)](#)

²⁶ [The 16 tests you need to perform on raw milk, finished products | 2016-06-16 | Dairy Foods](#)

²⁷ [Biosensing approaches to detect potential milk contaminants: a comprehensive review: Food Additives & Contaminants: Part A: Vol 38, No 7 \(tandfonline.com\)](#)

²⁸ [Tetra Pak Bactoffug Spore Removal Device | Tetra Pak Sweden](#)

Economic Impact

In numerous EU member states, including Ireland, Luxembourg, and Belgium, bimonthly milk deliveries to dairies have increased by up to 35% year over year after the elimination of milk quotas in 2015. Outside of Europe, milk production has grown during the past few years as well.²⁹

Ireland is one of the top producers of milk and dairy products per population. Dairy goods and ingredients made up one-third of all export categories and were worth more than €4 billion in 2018, according to the Bord Bia (Irish Food Board) annual report of Export Performance and Prospects.³⁰

An example for the economic consequences resulting from the late blowing defect is its impact on the Grana Padano PDO cheese production, where 15-35% of the total production was impacted by the spores. Their production value was 1.7 billion euros in 2022.³¹

3.2 Stakeholder Map

As previously mentioned, conditions on the farm influence the quality of the milk. The first and most crucial link in the dairy chain is appropriate feed, which helps to ensure good milk production and overall milk quality by preventing contamination of the milk. All water sources must also meet high quality standards. At the farm level, dairy farmers also pay close attention to cleanliness of milk production facilities, animal health, sensible use of antibiotics, milk equipment, milk production, and storage.^{32,33}

With regards to veterinarians, they perform routine inspections at the dairy farm. The farm's milking facilities and hygienic conditions are verified and monitored in addition to the health of the animals. Moreover, the responsible regional inspection association controls the producers, taking samples of raw milk several times per month. This guarantees that there are no inhibitors or other elements in the raw milk that would lower its quality.³⁴

The next group consists of stakeholders that are both involved in milk as well as cheese production. Firstly, this would be the transportation companies or milk collectors. The raw milk is pumped out of the cooling tank and into the milk collection vehicle after it has been examined for smell, color, and temperature. Milk samples are automatically taken at the same time and analyzed in a separate facility or laboratory.³⁴

When the milk is initially picked up from the dairy farm, professionally trained milk tanker drivers do the first quality check on it (looking for antibiotic residues, the milk's flavor, color, and temperature). Before processing, raw milk is checked for antibiotic residues; if the amounts are higher than allowed by law, processing is stopped. Following the quality checks at the farm

²⁹ [Frontiers | Detection and Enumeration of Spore-Forming Bacteria in Powdered Dairy Products \(frontiersin.org\)](https://www.frontiersin.org/journal/article/10.3389/fmicb.2023.1128423)

³⁰ [Occurrence and identification of spore-forming bacteria in skim-milk powders - ScienceDirect](https://www.sciencedirect.com/science/article/pii/S0950268823000000)

³¹ <https://news.italianfood.net/2023/04/25/grana-padano-pdo-sales-grow-thanks-to-exports/>

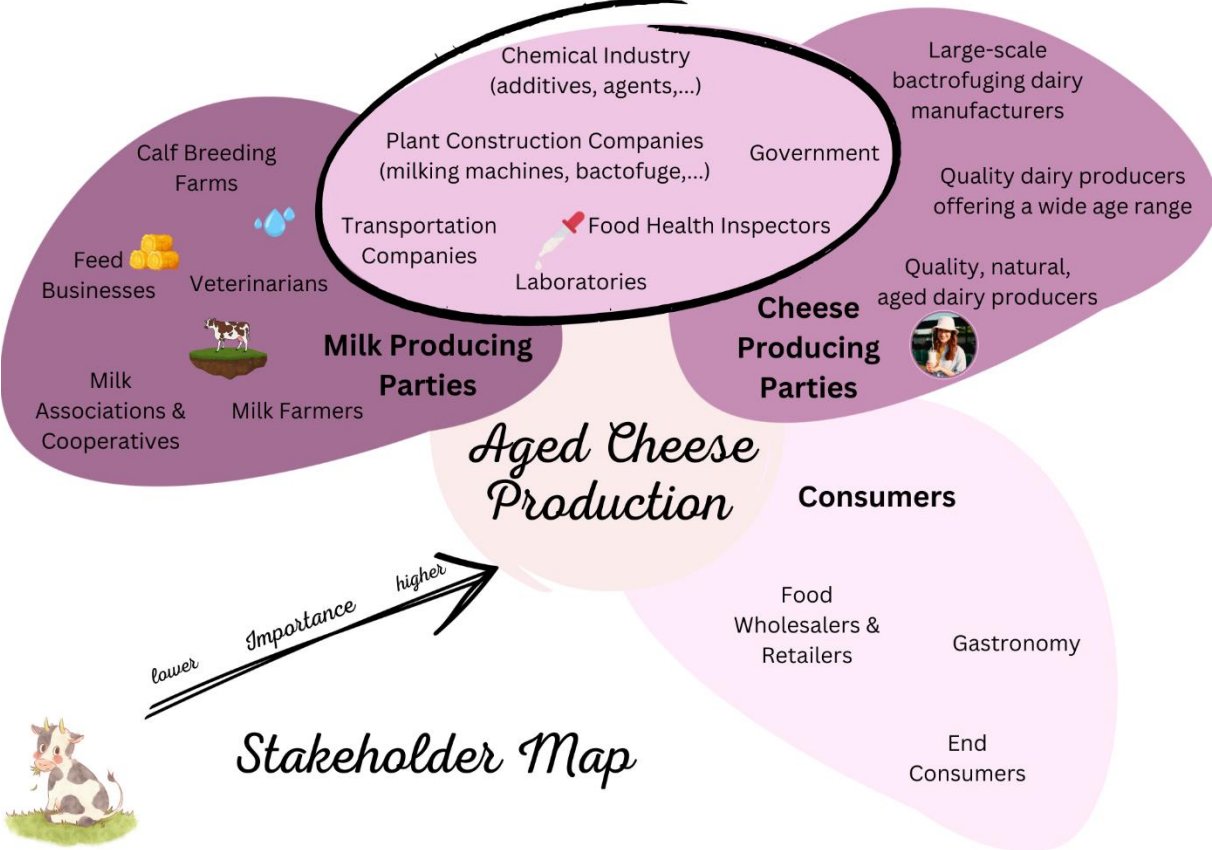
³² [Plakat_Weg_der_Milch2017_A1_web.pdf \(biosphaerenreservat-rhoen.de\)](https://www.biosphaerenreservat-rhoen.de/plakat_weg_der_milch2017_a1_web.pdf)

³³ [EDA Fact sheet on Food Safety - February 2020.pdf \(euromilk.org\)](https://www.euromilk.org/eda-fact-sheet-on-food-safety-february-2020.pdf)

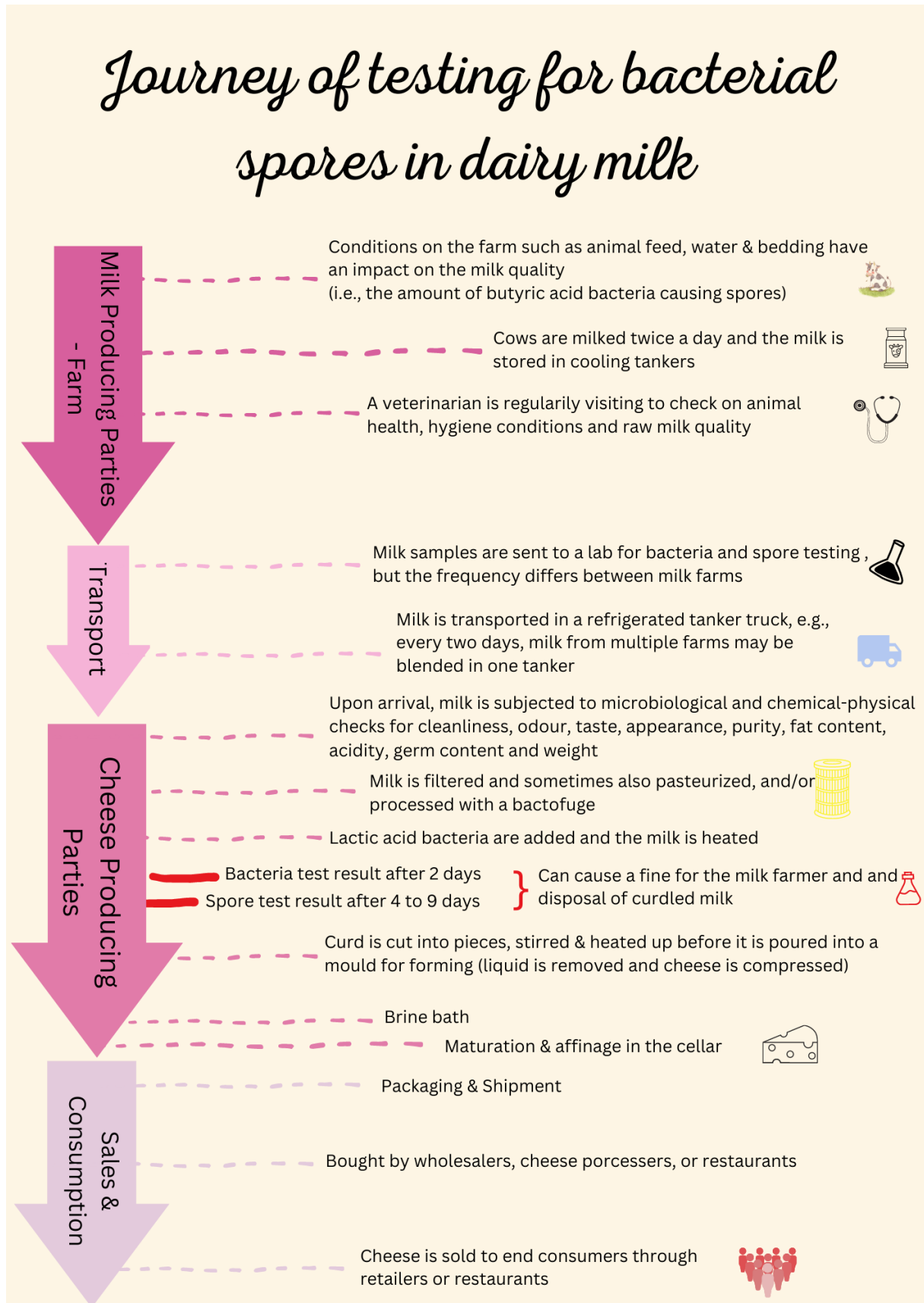
³⁴ [Fakten_Milch_September_2017_A4.pdf \(milchindustrie.de\)](https://www.milchindustrie.de/fakten_milch_september_2017_a4.pdf)

and during transport, specialized laboratories conduct tests on milk samples taken from milk tankers. These tests look at the contents of milk fat, milk protein, and other milk components, as well as cell count, bacterial count, contamination levels, antibiotics, and other parameters. All of these tests are conducted under the responsibility of dairy companies.³³

As a last group that is concerned with production in some sense are the cheese producing parties, that no matter their form examine the raw milk for cleanliness, odor, taste, appearance, purity, fat content, acidity, germ content, and weight using microbiological and chemical-physical methods.³⁴



3.3 Journey map



3.4 Personas

Greta Ekströmer



What is the story of our persona?

Greta makes aged cheese out of cow milk with natural characteristics. She frequently sends BAB-spore tests to laboratories to analyze the milk used in production. If the level turns out too high, Greta discards the cheese produced with the bad milk and halts production until she knows that the spore value is back on an acceptable level.

User	Need	Insight
Produces high-quality aged cheese from cow milk with natural characteristics.	A low Butyric-acid spore value in the milk to maximize her operational profits and minimize wasteful production.	<p>Cheese aged more than 5 months gets bad if there are high spore values.</p> <p>It takes five to nine days to test the spore level in the milk at a laboratory, as they do not work on weekends.</p> <p>Because of the delay, some producers need to discard production from the day the test was taken and halt production until they know that the spore value has returned to an adequate level.</p>

Hilda Zollitsch



What is the story of our persona?

Hilda Zollitsch oversees her family's Swiss cheese production. The family's cheese is a renowned delicacy that has been distributed globally through high-end restaurants and reputable cheese vendors for ages. Its intense flavor comes from the traditional manufacturing process, where no milk is bacto-fugated, and the cheese is aged for at least five months. To keep up with her high demand, Hilda uses multiple milk suppliers. Unfortunately, most milk farmers use silage fodder for their cows; which can lead to high concentrations of butyric acid spores, that damage aged cheese. Hilda can send spore tests to milk testing labs to address this issue, but if the milk is found to be highly spore-contaminated, all cheese made from that milk must be discarded until a better result is received. So, to avoid this hassle, Hilda has decided that all her suppliers must use hay fodder instead, even though this significantly increases her milk cost.

Hans Gruber



What is the story of our persona?

Hans Gruber is a fourth-generation Austrian dairy milk farmer. The market for cow milk is highly competitive, so it is hard to make a residual income. Still, Johan has found that he can differentiate slightly and increase the price per volume by increasing the milk quality. The quality is measured by its concentration of butyric-acid spores, which varies daily. Making the best possible spore prediction to optimize the price is crucial. As if it turns out that Johan sold his milk with a too-high quality classification, he needs to pay back a discount to the dairy that bought it. And on the contrary, if it turns out that he sold the milk with a too-low quality classification, he did not maximize the price he could have gotten for the milk.

3.5 Empirics

Interviewee 1, a cheese storage expert and vendor, 2023-04-25

Interviewee 1 works for a firm that buys and ages cheese, but they do not produce any cheese. However, they have noticed the problem of defects when cheese ages. Something they discuss with their suppliers on almost a daily basis. “What is happening in cheese Sweden!?” (Interviewee 1)

It is known that the cheese is sensitive to the bacteria/spores in the milk that affect the cheese during storage. But, according to Interviewee 1, you don't really know which bacteria you have, and how they affect the cheese. Problems with spores seem to come when you industrialize milk production. It changes over generations, some farmers haven't had a problem with spores for 25 years and then it comes out of a sudden.

Interviewee 1 gave us the tip to talk to someone who has a dairy, and gave us the following suggestions, to look for the source of the problem and areas where Unicorn DX could be used:

- Almnäs bruk (unpasteurized milk), they are very interested in what affects cheese and how to optimize production from a quality and profit perspective.
- Gäsene dairy, Ljung dairy (pasteurized milk).
- Skåne dairies have big problems.

Interviewee 2, an MD at a Swedish medium size dairy, 2023-04-25

According to Interviewee 2, there is a huge need for a fast and reliable test.

The buyer would probably be the bio-labs (if the biosensor is expensive), but it would still be because the dairies have a need. However, interviewee 2 would prefer if the dairy had the technology, otherwise it should be either the milk truck, or the ability to send tests daily for quick answers (like they do now, but faster).

After we told Interviewee 2 that the cartages could have the same cost at the farm as at the lab but that he would need a biosensor investment to do the test himself of approx. SEK 25 000, he said that he would take the cost himself if he was sure the test worked.

The big problem is with spore tests for butyric acid-forming anaerobic spores, not bacteria tests, because it takes a long time to get an answer. Currently, spores are cultivated in a lab. Bacteria test only take a couple of days so that is under control. The spore test takes 5 days, and up to 9 days on long weekends as the lab personnel only work on regular bank days. This time delay costs this dairy approx. SEK 100'000 a day due to spoiled cheese, or not to cheese okay cheese.

Big dairies use a bactofuge that reduces the spores. The problem is that the bactofuge destroys the quality of the cheese, but it doesn't matter much if the cheese is sold young (unaged).

Currently, this dairy makes a test per day, 365 days per year, as they need an early warning system. They test today for spores per liter. If the concentration is >250 they stop the production until the value is <110. Sometimes the spore test takes 7-9 days, on long weekends. Therefore, the delay is a huge problem. At Easter it cost the production SEK 400 000, because they got a 250+ result just before the easter weekend and it took an additional 4 or 5 days before they were notified that the value dropped below 110 again. In between, they sold the milk to a large Swedish diary that uses a bactofuge to minimize the loss.

The biosensor can thus save time and money to a great extent.

Interviewee 3, Quality and Sustainability Manager, at a Swedish medium size dairy, 2023-04-27

This dairy is legally an economic association operated collectively by 23 distinct farms. They make random samples of the cheese milk on each farms twice a week, making it 46 tests a week.

It takes 5 days to get a spore result from the lab under normal circumstances, and the lower the trace value, the higher the quality of the milk. Current lab tests detect at least 25 spores per liter, but values can be much higher, for example 1700 spores per liter.

Values of >200 spores per liter are considered alarming. However, the dairy does not throw away cheese, but they adapt production; Their cheese products are stored between 2 and 22 months. At higher values, they choose to produce the cheese products that have a shorter shelf life, i.e., that can be sold younger.

They are accurate at the farm level, and therefore rarely have high values. However, high values lead to fermentation in aged cheese for them as well. Therefore, the dairy receives a discount on the price depending on the quality, i.e., the test result. The milk farmer is thus paid based on the test results/quality of their milk.

A bactofuge can clear the milk of bacteria and spores, but it affects the taste. It becomes more sterile and less natural. For that reason, the dairy do not want to use a bactofug.

Eurofins Milk Testing Sweden AB in Jönköping does all the testing today. They are probably used by most dairies in Sweden. The price per test is SEK 50.

For the dairy it is important that the test is accredited, so according to Interviewee 3, they would send the tests to a lab even if the response time would be shorter and the tests would technically be feasible on site. However, Interviewee 3 still believes that the test would be useful at farm level, as it would be easier to plan sales. It would also be interesting for the dairy to plan production on a daily basis, i.e., what they should produce.

Interviewee 2, an MD at a Swedish medium size dairy, 2023-04-28

Follow-up questions to Interviewee 2:

- How much do you pay per test today?
- How much do late test results cost on average per year?
- What do you do with what you have stored from the time the test was sent until you received the test result?
- Are there any more farms like this that you can recommend?

Answers:

The test costs nothing officially, because it takes place under the auspices of the Skåne dairy at every pick-up, their many external customer.

It costs 70' per day to throw away curdled milk, and it affects about 4–5 days of production a year (approx. 400' SEK/year). In the event of a high test result, they throw away everything produced from the bad milk on the day the sample was taken until a result below 110 spores per liter is returned.

While they await a better result, they take the opportunity to do tasks that at some point still have to be done in the dairy, such as cleaning. They also sell the in-house produced milk for approx. 30% of the revenue value. In other words, they lose 70% of the revenue but have almost 100% of the costs if there are no meaningful tasks to perform.

It is only this dairy that produces cheese in this way (unbactofuged aged cheese), at least in this size class in Sweden. Magnus, the MD at Gäsene dairy, may possibly be another actor Spores only become a problem after 5 months. But there are still many tailor-made producers in the world with 400–500 cows. In Schweiz several hundred, the same in France, England, etc.

If you use hay instead of silage, the problem with spores disappears almost completely. Many Swiss producers therefore prohibit suppliers from using silage. Therefore, they also pay more for the milk, but it pays off as it is enough that one out of perhaps a hundred suppliers would be careless before it would become indirectly more expensive to purchase silage-foddered cow-milk. The problem is that you do not have hay using suppliers in Sweden.

Interviewee 4, an organic cheese producer from Austria, 2023-04-28

The organic cheese dairy has specialized in the production of cheese specialties such as Gouda, Camembert, red cultures and more, processing only sheep, goat and cow's milk from organic farming.

I don't see the BAB spores in the milk as a problem, as they mainly occur in silage feed. Therefore, this problem is more common with cow's milk, and less common with sheep's and goat's milk, which I mainly use. Milk contamination can also come from the udder, although this is also mainly a problem with cow's milk, as sheep and goats have hard faeces.

Furthermore, I use a lot of hay milk for my cheese production, whereby the BAB spore problem is very low from the ground up. In organic production, no additives are allowed, but I still have no problem with spores.

For the reasons mentioned, I do not test my milk before starting cheese production. I work on the assumption that no spores are present. Apart from that, I work in long-standing and good cooperation with my milk suppliers, who always provide me with high-quality milk.

If it does happen that spores were in the milk, the cheese production unfortunately has to be thrown away completely.

Interviewee 5, an organic cheese producer from Austria, 2023-05-02

Since I mainly make soft cheese and fresh cheese, I don't have much of a problem with BAB spores. Late-blowing is mainly a danger with semi-hard and hard cheeses, and here too the main contamination comes from the cows' silage feed.

I do not test the milk before production starts at the moment. Fresh cheese production is very transparent, so if there is any contamination during production or if there is an inflammation of the udder that has contaminated the milk, you would notice it immediately the next day in the cheese itself and can then act accordingly (throw the cheese away). Soft cheeses that have a ripening period of only 3 months are therefore not affected. However, it must be said that blue cheese can also have a ripening period of 6 months, which falls within the critical period.

I don't do milk tests per se, but the hygiene standards are checked several times a year by the food control. I also carry out several self-checks.

Other

We tried to contact large-scale dairies and a testing laboratory as well, but we could not get in touch with their managers/experts. However, a lab-staffer we talked too at one of Sweden's large-scale dairies confirmed that they took samples which they sent to external laboratories for testing, and that it took days for them to get a result. Unfortunately, she did not know anything about the cost or consequence caused by the test result.

3.6 Other investigated components within milk - Salmonella

Humans get salmonella commonly through the consumption of food that has been contaminated, especially egg, meat or dairy products. The illness-causing bacteria level is 100.000 or more, which can be reached rather quickly when food is not stored properly as the reproduction period at room temperature is 20minutes only. Symptoms of salmonella can be nausea, diarrhea, fever or vomiting, lasting up to a few days and hence imposes a risk on human health and wellbeing.³⁵

Testing for Salmonella in the dairy industry is an important topic, especially since contamination can occur at any point along the value or production chain. The decreasing trend in salmonella cases in Europe that has been observed between 2008-2016 has come to a halt, making it a continued pressing issue.³⁶

→ a fast testing method would be needed

→ societal relevance would be given

³⁵ [Salmonellen - AGES](#)

³⁶ [Phages for biocontrol in foods: What opportunities for Salmonella sp. control along the dairy food chain? - ScienceDirect](#)

4. Food & Beverages – Wine

4.1 Information

What is the problem?

- Fungi can cause spoilage of wine.
- Some fungi can cause off flavours and aromas that would affect the quality of wine.
- Fungi can grow in wine after it has been bottled, leading to spoilage and reduced shelf life.
- Some fungi can produce mycotoxins, which can be harmful to human health.

What is the need?

- Need to detect fungi before, during and after the wine fermentation.

Why?

In the wine industry, it is important to detect fungi for several reasons:

1. Quality control: Fungi can grow on grapes during the winemaking process and produce off-flavors and aromas that can affect the quality of the wine. By detecting fungi early in the winemaking process, winemakers can take steps to prevent spoilage and ensure the quality of the wine.
2. Preservation: Fungi can also grow in wine after it has been bottled, leading to spoilage and reduced shelf life. By detecting fungi in bottled wine, winemakers can take steps to prevent spoilage and ensure that the wine has a longer shelf life.
3. Health and safety: Some fungi can produce mycotoxins, which can be harmful to human health. By detecting fungi in wine, winemakers can ensure that the wine is safe for consumption.
4. Regulatory compliance: The wine industry is subject to regulations regarding the presence of certain fungi, such as *Brettanomyces*, which can produce off-flavors in wine. By detecting these fungi, winemakers can ensure compliance with regulatory requirements.

What can Unicorn DX do?

- With the help of its sensitive Bio-nanosensor, Unicorn DX can detect the presence of fungi that can spoil wine with high accuracy. These fungi include genera such as *Hanseniaspora* (anamorph *Kloeckera*), *Metschnikowia*, *Candida*, *Pichia*, and *Issatchenkia*, as well as filamentous fungi such as *Botrytis*, *Cladosporium*, and *Aspergillus*.^{37 38 39}

³⁷ Abdo H et al. (2020). Colonization of wild *Saccharomyces cerevisiae* strains in a new winery. *Beverages* 6:9.

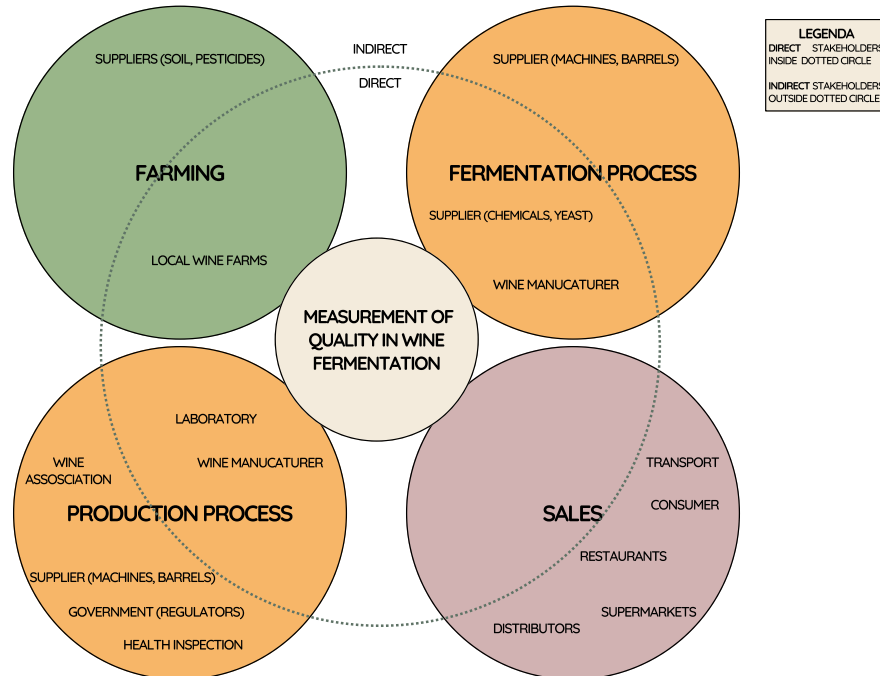
³⁸ Fleet GH. 2003. Yeast interactions and wine flavour. *Int J Food Microbiol* 86:11-22.

³⁹ Jolly NP, Varela C and Pretorius IS. 2014. Not your ordinary yeast: Non-*Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Res* 14:215-237.

Reason for dropping?

We were facing a challenge of limited availability of potential interviewees within a short time frame.

4.2 Stakeholder map



Farming

- Local wine farmers
 - Responsible for delivering the supply of grapes to larger wine producers or for their own production.
- Suppliers of farming material
 - Grapes can be affected by the soil or products in the soil, that could cause fungi.

Production process⁴⁰

- Laboratory
 - Larger wine manufacturers with laboratory on site to actively monitor the wine production and research new wines.
- Wine manufacturer
 - Most important stakeholder in the problem of measuring the fungi developments in wine production.
- Wine association
 - Indirect stakeholder that monitors if wine is produced safely and responsibly.
- Supplier of machinery

⁴⁰ Regecova et al. (2022) Detection of Microbiota during the Fermentation Process of Wine in Relation to the Biogenic Amine Content. <https://www.mdpi.com/2304-8158/11/19/3061>

- Indirect stakeholder that is involved in the process of production with supply of machinery and storage barrels. Barrels are the location where fungi can operate after fermentation.
- Government
 - Setting the guidelines for wine production and monitoring safety in the industry.
- Health inspection
 - Making sure the produced wine is according to health regulations and safe for consumers.

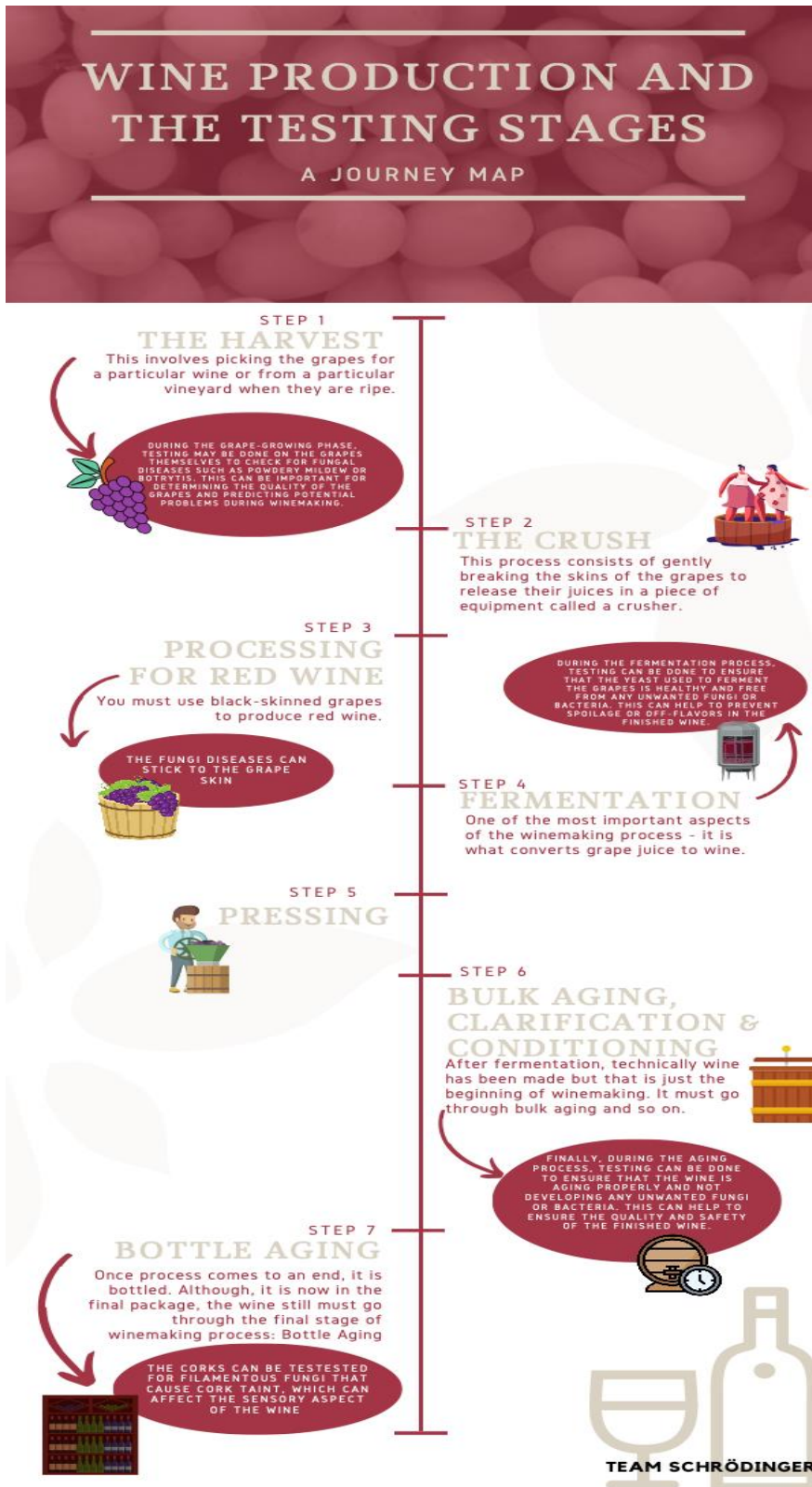
Fermentation process

- Supplier of fermentation product
 - Usage of yeast in the production of wine is needed for more consistent quality in wine product. Bad supply can be cause for negative effects of yeast fungi in fermentation.
- Wine manufacturer
 - Core stakeholder in the fermentation process. Most interest and influence in measuring the fungi in wine fermentation.
- Supplier of machinery
 - Indirect stakeholder that is responsible for fermentation tanks.

Sales

- Transport
- Consumer
- Restaurant
- Supermarket
- Distributors
 - All indirect stakeholders that negatively experience when the product is influenced by bad fungi in wine production. Can cause health risks and food waste.

4.3 Journey map



4.4 Persona

PERSONA 1

Jean- François Vineyard Owner

Jean-François is a winemaker who uses natural methods to cultivate and ferment the grapes, resulting in a unique and organic flavour profile. Biodynamic wines are becoming increasingly popular due to their ethical and natural production methods. They also use natural preparations and sprays to improve soil health and prevent diseases. For this reason, Jean-Francois must have access to precise measurement techniques and a laboratory for testing his vines and wines.



Team Schrödinger

PERSONA 2

La Société de Thauvin

La Société is a French wine manufacturer. Originally founded in 1994 as a small hobby business for the family Thauvin, it quickly grew out to be a leading wine producer in the Bordeaux region. Mainly producing red wines like Cabernet Sauvignon. The company has industrialized their production process by installing large machines, brewing tanks and even a laboratory to create and test new wine combinations. This has been the key reason for their quick growth in recent times. La Société does not only rely on their own grape farms but has partnerships with selected regional grape farms. Collecting grapes from all these different farms has not gone without issues. Grapes naturally have yeast on their outside layer and having all these different grapes together, from different soils and area can create difficulties during the fermentation process. Maxime Thauvin has had to throw away more and more tanks full of fermented wine, because of the increasing diversity in suppliers. Around one 1.000-liter tank every week gets rejected for bottling. With a projected growth in the coming years and more supplier partnerships Maxime wants to be ahead of the spoilage problem because they are very much against unnecessary food waste. Maxime believes in a sustainable economy and is proactive in the battle against food waste. La Société wants with full insight in their supply chains and build relations with foodbanks to minimize their waste of food in the production process.



User

Manufacturers of wine product

- Small, bio dynamic
- Large, modernised

Need

- Quick and correct measurement
- Active monitoring

Insight

- Quick and correct measurement
 - Ability to measure on site in order to have quick results and be able to act on it.
- Active monitoring
 - Reducing wine waste at early stages, before it has to go into storage process

5. Other Food & Beverages

5.1 Fruit juice

What is the problem?

In manufacturing of fruit juices researchers have found three new bacteria of the Alicyclobacillus genus: *A. mali*, *A. fructus* and *A. suci*. One of these species, *A. suci*, produces guaiacol, which creates a medicinal, smoky or rubber-like flavor in shelf-stable apple juice.⁴¹ Resulting in food spoilage due to late detection, after consumption. Our idea was to create a test before juice is bottled and shipped out.

Seen in fruit juice, mainly apple, orange, passion fruit, pear, watermelon, banana, lemon and grape. With 6,1% of 180 tested juices in a sample test impacted.⁴²

What is the need?

Needed is a tool that is able to measure the fruit juice before packaging and give results on short term basis. Its detection is a challenge for the quality control departments, because it takes several days to get the results of traditional microbiology methods.⁴³

Despite the high prevalence of Alicyclobacillus in specific months of the harvest season, the overall counts found in the passion fruit juices were low (between 1.1 and >23.0 spores/100 mL)⁴⁴

What can Unicorn DX do?

Unicorn DX technology has the ability to measure nano particles in small liquid samples. Doing so it should be able to detect the bacteria in the fruit juice product after pasteurization. And because of the ability of Unicorn DX to get a quick analysis within a few minutes, it could help production companies to get results before shipping the product. Which would save them money but also bad customer experiences.

Reason for dropping?

The topic has only been researched at surface level, and finding real used current methods was difficult in order to realistically compare it to Unicorn DX. Other ideas were more relevant and with clearer problem in the journey map, that it made sense to drop this idea.

⁴¹ <https://www.labcompare.com/617-News/579774-Bacteria-That-Makes-Apple-Juice-Go-Bad-Taste-Weird-Identified/>

⁴² <https://pubmed.ncbi.nlm.nih.gov/21356453/>

⁴³ <https://pubmed.ncbi.nlm.nih.gov/28800823/>

⁴⁴ <https://pubmed.ncbi.nlm.nih.gov/21356453/>

5.2 Restaurant & Hotels

Due to the quantity/variety of food offered and the propensity for food to be kept at room temperature for extended periods of time, buffets can provide a number of dangers. The 'self-serve' form of buffet dining also highlights the different levels of personal cleanliness that diners may possess; once one customer has touched the food or used utensils without washing their hands, suddenly everyone else may be at risk. Typical bacteria that are present in buffet food or in areas where food is served include E.Coli, Salmonella, Listeria.⁴⁵

The four most important considerations for proper food hygiene are known as the "4 C's": Cooking, Cleaning, Chilling, Cross-contamination, which also constitute the current technologies used to manage the aforementioned bacteria on hotel & restaurant buffets.^{46 45}

Temperature measurement for cooking and cooling: To ensure that food is prepared to a temperature hot enough to kill bacteria, use a food thermometer. When food is in the "Danger Zone," which is between 40°F and 140°F, germs that can make you sick swiftly proliferate. Further, any perishables on buffets should only be left out for a maximum of two hours (2-hour rule).⁴⁵⁴⁷

→ Summary: Restaurants and hotels are two places where many people gather daily. They meet for business events or family experiences, during these periods people from different areas come in to contact with each other. Food hygiene practices at these events and buffets has to be monitored. To mitigate risks, allergens and safety of the people. Therefore, we thought there to be a need to measure different foods for possible infection or bacteria that can be harmful in order to provide a safe and healthy experience for the customer and other people involved with the situation.

→ However, it became clear quite quickly that very fast and efficient procedures for buffets, like the 4C-rule, are well established and working, hence the decision was made to not further pursue this topic.

⁴⁵ [Food hygiene practices at buffets and events | Risks, allergens and safety \(cpdonline.co.uk\)](#)

⁴⁶ [Salmonella and Food | CDC](#)

⁴⁷ [Food Safety for Buffets and Parties | CDC](#)

5.3 Sugar cane

What is the problem?

Sugar canes are a natural product, often grown and used in Asia. The sugar cane is cut down and harvest with the goal of using the juice from the sugar canes to make sugar. This process takes cleaning, crushing, clarification, evaporation, crystallization and refining of the juice. Disease observed in sugarcane plant is a big challenge and danger to farmers that has a financial impact if not detected on time⁴⁸.

What is the need?

To accurately measure the sugar cane juices for possible harmful bacteria that cannot be detected from the outside of the sugar cane plant.

Disease observed in sugarcane plant is a main challenge and danger to farmers that triggers a financial impact, it is sugarcane yield and manufacturing if now no longer detected on time. There is a detrimental impact at the economic system if the cultivation of those vegetation decreases. Sustainability of manufacturing could be very critical within the control of enter sources along with seed, water, soil, and pesticides. Crop manufacturing and upkeep will drop aggressive flexibility if the vegetation get harm as they grow. Detecting and spotting sugarcane sicknesses has performed an essential function in such sicknesses are unavoidable.^{49 50}

What can Unicorn DX do?

Unicorn DX has the technology to take small samples of liquids, in this case the sugar cane juice and analyse the liquid sample for possible nano particles present in the liquid. This can be helpful in order to spot harmful bacteria or viruses inside the sugar can juice, before going into the production process.

Reason for dropping?

The Unicorn DX method would financially not be viable for the users of the sugar cane problem, that in the current state of the technology there is not further point of continuing research. The problem was not in depth explored and thus is chosen for other problems that look more realistic.

⁴⁸ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8468687/>

⁴⁹ Grisham MP, Johnson RM, Zimba PV. Detecting Sugarcane yellow leaf virus infection in asymptomatic leaves with hyperspectral remote sensing and associated leaf pigment changes. *J Virol Methods*. 2010 Aug;167(2):140-5. doi: 10.1016/j.jviromet.2010.03.024. Epub 2010 Mar 31. PMID: 20362003.

⁵⁰ Ratnasari et al. (2022) <https://ieeexplore.ieee.org/document/9776685>

6. Water

Table 1
Summary of water quality indicators commonly combined with FCM analysis.

Method/indicator	Description	Advantages	Disadvantages
Heterotrophic plate count (HPC)	Culture-based measurement of the heterotrophic microorganism population in a water sample.	<ul style="list-style-type: none"> Widely employed, so provides a common basis of comparison to many studies. Confirms the presence of viable bacteria. 	<ul style="list-style-type: none"> Takes multiple days to deliver results. High variability. Limited detection capacity—only about 1% of bacteria in drinking water are detectable through HPC.
Epifluorescence microscopy (EFM)	Illumination of a sample from above with fluorescent light, enabling visual inspection of particle characteristics that cannot be detected through traditional optical microscopy.	<ul style="list-style-type: none"> Useful validation tool, since good agreement has been reported between EFM- and FCM-based TCC and ICC results. Provides additional information about factors such as cellular morphology, cellular damage, and staining efficacy that can aid interpretation of FCM data. 	<ul style="list-style-type: none"> Time-consuming and labor-intensive. Highly subject to human error.
Molecular techniques	Techniques such as PCR, DNA sequencing, and gel electrophoresis.	<ul style="list-style-type: none"> Can identify specific microbial strains present in a sample. Particularly valuable when a strain cannot be specifically stained by a fluorescent antibody or other marker. Provides deeper insight into the differential impacts of water-treatment processes on various microbial classes (e.g., PAOs and GAOs). 	<ul style="list-style-type: none"> Does not distinguish between viable and non-viable microorganisms. Limited potential for online analysis.
Adenosine triphosphate (ATP)	Measurement of ATP—the “energy currency” of a cell—through extraction and reaction with a bioluminescent complex.	<ul style="list-style-type: none"> Offers a fast, simple, and cost-effective indication of the overall level of viable microbes in a sample. 	<ul style="list-style-type: none"> Does not provide viability information at the single-cell level. Measurements can be confounded by the presence of free ATP and other interfering compounds.
Assimilable organic carbon (AOC)	Assay that provides an indication of biological stability (i.e., the inability of water to support microbial growth).	<ul style="list-style-type: none"> Highly relevant to drinking water treatment. 	<ul style="list-style-type: none"> Conventional assay is time-consuming. Use of pure cultures of test organisms in the conventional assay may be an imperfect proxy for real-world conditions. Use of mixed cultures may yield more realistic but less consistent results.

Figure 1: Safford, H.R., & Bischel, H.N. (2019) Flow cytometry applications in water treatment, distribution, and reuse: A review. *Water Research*, 151(15), 110-133.

6.1 Wastewater

What is the problem?

In the biological sanitation process bacteria are used to sanitise the water.⁵¹ The problem is that bacteria can be sensible for chemicals in the polluted water.⁵² Our idea was, therefore, to proactively check if there are enough bacteria in the treatment water before the treatment process is too significantly impaired, leading to pollution of our lakes and seas, which could danger both humans and other animals in our ecosystem.

What is the need?

Interview (2023-04-11)

We talked to two municipality employed water-testing biologists. They use microscopy once a week to check the biomass for activated sludge, what types, filaments (some good/less good). They receive a result in an hour or two. The test has more to do with checking the structure than detecting specific bacteria. There are biosensors used for water quality testing, but it does not seem to be commonly used.

What can Unicorn DX do?

⁵¹ <https://www.fluencecorp.com/what-is-biological-wastewater-treatment/>

⁵² <https://www.foretagarna.se/nyheter/riks/2022/januari/biltvatten--misstag-och-tips-du-bor-kanna-till/>

Our idea may thus be feasible as an early warning system. For instance, if mass-produced the cost could allow more frequent search for a specific bacterium, as a complement to the current microscopy and it would be even better if we reengineered it for continuous testing.

Reason for dropping?

As a sensor, it would fail to completely capture the biofilm's internal environment, which could have caused resulted in crucial dynamics being overlooked.⁵³ For instance, it does not check the structure, so it is not a perfect substitute, and if we want to count particles continuously and in huge volumes, other tech as flow cytometry may be even more feasible as a complement to microscopy. Also, Wastewater treatment is generally not as detailed as e.g., drinking water. Therefore, we were recommended to investigate drinking water usage instead by the biologists at the Swedish municipality.

⁵³ <https://www.sciencedirect.com/science/article/pii/S2666053921000199>

6.2 Drinking water

What is the problem?

Drinking water needs a more detailed and specific search than wastewater. (Interview with the lab personnel at a Swedish municipality). This is because pathogens cause vast problems, both in developed and developing countries. For instance, it has been estimated that pathogens present in drinking water lead to around 4 to 33 million cases of gastrointestinal illness in the United States each year, costing the society at least \$1 billion⁵⁴.

The problem in developing countries is that 1/3 people lack safe drinking water, according to WHO/UNICEF 2019. More than 2 billion use feces-contaminated drinking sources. However, even though the problem is vast, drinking water in developing countries are rarely tested as old/current testing methods are too difficult, impractical, and costly.⁵⁵

What is the need?

A fast, reliable, and cheap test.

The municipality we interviewed (2023-04-11) used bacteria and chemical tests and had just invested in flow cytometry as a complement. The bacteria and chemical test are used once a week and it takes 1 to 7 days to get a result. It checks for:

- E Coli
- Cultivable slow growing bacteria (e.g., from stagnant water)
- Microfungi
- Coprophagia (viruses attacking bacteria)
- Parasites

Home wells faced much of the same problems as the municipality drinking water plants. However, 1) The consequence of water contamination is lower as it only affects the humans and animals drinking from the specific well, and not thousands of humans. 2) Expensive high-tech equipment for water tests are not feasible because of their costs in relation to their impact. Therefore, they are only tested every three years or because of an event in the surrounding environment or because of a strange smell and/or taste.⁵⁶ It takes about 10 working days before the analysis result is ready and sent to your home by post.⁵⁷

Normal home well water tests check the following⁵⁸

- E coli
- Coliform bacteria
- Number of microorganisms at 22 °C

⁵⁴ <https://www.sciencedirect.com/science/article/pii/S0043135418310339>

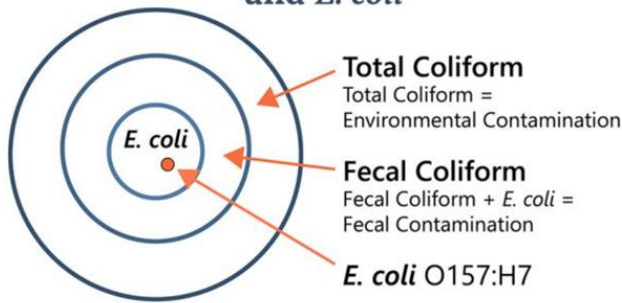
⁵⁵ <https://www.aquagenx.com/project/developing-countries/>

⁵⁶ <https://www.livsmedelverket.se/livsmedel-och-innehall/dricksvatten/egen-brunn2/vattenprov-och-analys-av-ditt-dricksvatten>

⁵⁷ <https://callidus.se/vattenanalys/>

⁵⁸ <https://www.livsmedelverket.se/livsmedel-och-innehall/dricksvatten/egen-brunn2/vattenprov-och-analys-av-ditt-dricksvatten/normal-analys>

Total Coliform, Fecal Coliform, and *E. coli*



59

E. coli or coliform bacteria in water signals contamination from fecal matter of humans or animals. Coliform bacteria will not likely cause illness by themselves⁶⁰. However, the concentration indicates that other harmful ⁶¹[OBJ].

E Coli	Risk level	Sweden
<1 / 100ml	No risk	Acceptable
1–10 / 100ml	Low risk	Unacceptable /Warning
11–100 / 100ml	Intermediate risk	Unacceptable /Warning
>100 / 100ml	High risk	Unacceptable /Warning

Coliform bacteria	Sweden
<1 / 100ml	No contamination
1-50 / 100ml	Acceptable for home wells
>50 / 100ml	Unacceptable

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What can Unicorn DX do?

Unicorn DX could provide a Fast, Frequent, and Reliable test with low alternative cost.

- Industrialized (automated) or Remote (sampling)
- Substitute (main test) or complement (e.g., warning system)

⁵⁹ https://www.researchgate.net/figure/Figure21-Total-Coliform-Fecal-Coliform-and-E-coli-WSDH-2010_fig1_340091889

⁶⁰ <https://doh.wa.gov/community-and-environment/drinking-water/contaminants/coliform>

⁶¹ <https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2022/1-2022-nr-14-e.-coli-och-koliforma-bakterier-i-dricksvatten.pdf>

⁶² <https://www.livsmedelsverket.se/globalassets/publikationsdatabas/rapporter/2022/1-2022-nr-14-e.-coli-och-koliforma-bakterier-i-dricksvatten.pdf>

⁶³ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7313150/>

For instance, flow cytometry, is probably too expensive for home use, and not remote enough. It could also be used as a complement at water plants to test for specific harmful microbes that are hard to detect with current methods that cause significant societal issues such as the E Coli and other coliform bacteria, as it is more sensible than flow cytometry. Also, if mass-produced, it could become a practical, simple, and cheap solution for developing countries.

Reason for dropping?

The concentration levels were too low for the field to be prioritized after the midterm presentation. This is as Occam DX tests an ml of liquid, not milliliters'.

Also, for drinking water plants there could be a higher need for permanent installations and more frequent and automated testing.

However, manual testing could also provide a good substitute or complement to current tests, as it is faster. The biosensor could also still function as a warning complement to slower established tests, as it relatively easy to test for 100 E-Coli / 100ml or high levels of coliform bacteria, both indicating a high risk.

6.3 Recreational water

What is the problem?

E Coli and Intestinal Enterococci indicates contamination in recreational water, which can make people sick.^{64,65,66}

What is the need?

Besides keeping people healthy, societies must follow regulations like the blue flag beach criteria which builds upon the EU Bathing Water Directive from 2006, and the recommendation of the World Health Organisation. The programme mandates a 95th percentile compliance with the limit values below.⁶⁷

Parameter	Coastal and transitional waters Limit values	Inland waters Limit values
Escherichia coli (Faecal Colibacteria)	250 cfu/100 ml	500 cfu/100 ml
Intestinal Enterococci (streptococci)	100 cfu/100 ml	200 cfu/100 ml

What are the current testing methods?

Culture-based methods for enumerating faecal indicator bacteria

- Advantages: affordable, highly standardized, easy to operate
- Limitations: time-consuming and requires additional time (~24–48 h) to produce results due to the need for incubation and growth of the target microbes

Culture-independent methods (real-time polymerase chain reaction)

- Advantages: higher specificity & speed
- Limitations: pre-processing steps such as filtration and nucleic acid extraction⁶⁸

What can Unicorn DX do?

It could provide faster, more precise & remote analysis for fecal indicator bacteria, and in relation to drinking water the concentration levels are more align with the current technical limitations of 1 ml samples.

Reason for dropping?

After the midterm presentation we opted for the food industry. Hence, Recreational water testing may still be a feasible application for Unicorn DX.

⁶⁴ <https://www.epa.gov/sites/default/files/2015-10/documents/rwqc2012.pdf>

⁶⁵ <https://thewaterproject.org/water-scarcity/health-implications-of-e-coli#:~:text=Diseases%20acquired%20from%20contact%20with,When%20E.>

⁶⁶ <https://www.epa.gov/national-aquatic-resource-surveys/indicators-enterococci#:~:text=Enterococci%20are%20typically%20not%20considered,protozoa%20may%20also%20be%20present.>

⁶⁷ <http://www.blflagg.org/wp-content/uploads/2016/04/Beach-Criteria-and-Explanatory-Notes-2015.pdf>

⁶⁸ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8196636/>

7. Chemicals

7.1 Paint

What is the problem?

Paint is produced by mixing pigments with resin, solvent and other additives. This creates the correct colour but also the quality and fluidity of the paint. Solvents are used to dissolve or change the physical properties of the paint. Solvents need to be measured in order to create the correct quality paint ⁶⁹.

What is the need?

Measuring the right level of solvent and avoiding particles inside the solvent to keep it pure and high quality of the product intact.

What can Unicorn DX do?

Unicorn DX is able to take small liquid samples of a fluid, like in this case they would be able to do with paint. In order to give a quick analysis of the sample test that could be used to prevent paint mixture going in to process of packaging. It can be a quick and efficient way to control the quality of the product.

Reason for dropping?

There was no real need or problem inside the paint producing industry currently to fix this. This makes it hard to find a possible market of interested company's and thus not financially attractive for the Unicorn DX organisation to continue further research.

⁶⁹ <https://www.keyence.eu/ss/products/microscope/vhx-casestudy/chemistry/coating.jsp>

7.2 Toothpaste

What is the problem?

Diethylene glycol (DEG), an adulterant in glycerol in toothpaste. Helps toothpaste from drying out, gives consistent texture and makes it glide out of the tube but DEG causes severe systemic and neurologic complications, including coma, seizures and hepatorenal failure ⁷⁰. In 2008 hundreds of children died from fake Colgate too much DEG⁷¹.

What is the need?

Limiting health risks of DEG in toothpaste, as it can be dangerous for young children and people with liver or kidney diseases. ⁷²

What can Unicorn DX do?

Detecting and measuring the toothpaste paste, or even before when materials are still liquid. Option to take samples and analyse them with results in short notice, should make it easier for manufacturers to take action on their bad batch of product.

Reason for dropping?

Option seems too farfetched and difficult to get accurate information about. Besides doubt about the possibility of measuring toothpaste paste, as it is thicker than normal liquid measured by Unicorn DX.

⁷⁰ <https://pubmed.ncbi.nlm.nih.gov/18414832/>

⁷¹ <https://www.securindustry.com/cosmetics-and-personal-care/fake-cologate-toothpaste-in-panama-contains-harmful-deg/s106/a10835/#.ZFaFg-xByw4>

⁷²

<https://www.medsafe.govt.nz/hot/alerts/chinesetoothpaste/dgstatement.asp#:~:text=Symptoms%20associated%20with%20consuming%20diethylene.kidney%20disorders%2C%20seizure%20and%20coma.>

7.3 Battery electrolyte

What is the problem?

Batteries are produced with an electrolyte solution, an acidic liquid used to transfer ions back and forth to charge the battery. Electrolyte need to be measured for purity to make the battery to work efficiently. ⁷³ We looked in to Redox Flow Battery, uses 2 tanks of liquid instead of minerals. Need to be tested in order to prevent corrosion inside the liquid. ⁷⁴

What is the need?

Quality control of electrolyte solution for batteries, that could be done on a quicker and cheaper method. Batteries that include more liquid based electrolyte solution, like the redox flow battery could benefit from more pure solutions.

What can Unicorn DX do?

The goal of Unicorn DX technology is to measure the electrolyte solution for impurity. By taking samples either manually or in an automatic way, to continuously monitor the impurities and avoid corrosion particles in the electrolyte solution. The purer it is, the better and more efficient the battery.

Reason for dropping?

There are more effective measurement tools on the market, according to Unicorn DX. Difficult to implement the use of Unicorn DX technology in the production process of battery electrolyte, as taking samples manually is a big waste of time and brings high cost that are hard to justify.

⁷³ <https://www.mdpi.com/2313-0105/9/3/164>

⁷⁴ Interview with battery researcher

7.4 Class cleaner

What is the problem?

The liquid of glass cleaner needs to be of high quality in order to effectively offer the service they claim to do. Nano particles of silica in the glass cleaner can reduce the quality of the product and the anti-fog ability. Also silver particles are regulated and have certain health risks, that they need to be monitored ⁷⁵.

What is the need?

Measuring of possible nano particles in glass cleaner liquid like silver or silica, to be used for quality control of the product.

What can Unicorn DX do?

Unicorn DX technology has the ability to find specific nano particles in liquid. In this case we would like to use the technology to measure the nanoparticles in the glass cleaner liquid and detect traces of these particles. Finding out the negative particles at early stages of production can ensure the quality of the product.

Reason for dropping?

There were better options available at the time of surface level research into the topic of glass cleaner. It was difficult to find currently used techniques in the production of glass cleaner and what their weaknesses are compared to Unicorn DX strength. Thus, has a decision been made not to continue further research into the topic.

⁷⁵ <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3741016/>

7.5 Detergent

What is the problem?

Detergent needs to be of high quality in order to perform the jobs that it is hired for. Detergent uses ethylene oxide (EO) as intermediate in the production⁷⁶. EO needs to be pure and tested on its purity in order to prevent safety hazards^{77,78}.

What is the need and what can Unicorn DX do?

Ability to detect traces of EO in the mixture used to create detergent during production. By samples of liquid tested it could measure the amount and specific types present. In order to make sure the purity is at >95%⁷⁹.

Currently done with high-performance liquid chromatography⁸⁰, nuclear magnetic resonance⁸¹ or gas chromatography⁸².

Reason for dropping?

While it can be found in liquid samples, most of the time it is gas based. Which is not something this technology could measure. Making it hard to find a market of interested companies and then we opted for more interesting markets.

⁷⁶ <https://www.chemicalsafetyfacts.org/chemicals/ethylene-oxide/>

⁷⁷ <https://www.mindthescience.org/chemicals-in-products/ethylene-oxide>

⁷⁸ <https://nj.gov/health/eoh/rtkweb/documents/fs/0882.pdf>

⁷⁹ <https://pubchem.ncbi.nlm.nih.gov/compound/Ethylene-oxide>

⁸⁰ <https://www.sciencedirect.com/science/article/abs/pii/S002196730087898X>

⁸¹ <https://onlinelibrary.wiley.com/doi/10.1002/pol.1974.180121210>

⁸²

https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/applications/application_note/14021/an_01-00139-en.pdf

8 Minerals – Detection rare earth metals

What is the problem?

Rare earth metals are valuable minerals that should not be wasted. They are critical for sustainability, but it is not sustainable to mine them. Hence, we need to recycle as much as possible⁸³

What is the need and what can Unicorn DX do?

Ability to detect or test for traces of rare earth metals in e.g., metal-containing wastewater at recycling plants. It seems like it is possible to test for traces of rare earth metals as they stick to cyanobacteria⁸⁴.

Unicorn DX could then probably be used to take samples of wastewater or other water sources, in order to check for rare-earth particles of minerals in the water.

Reason for dropping?

Even if the recognition element in Unicorn DX binds to rare earth metals, the binding interaction may be so small for the technology to pick it up the detection⁸⁵. Therefore, we opted for other markets that seemed more efficient and practical.

⁸³ <https://getrepowered.org/rare-earth-metals-recycle-technology/>

⁸⁴ <https://www.frontiersin.org/articles/10.3389/fbioe.2023.1130939/full>

⁸⁵ Interview with Occam DX, 2023-04-18