



The limits of testing for microbiological food safety

Prof. dr Han Joosten

Inaugural lecture upon taking up the post of Special Professor of
Food Safety Microbiology at Wageningen University on 27 February 2014



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ISBN 978-94-6173-793-9

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Meneer de Rector Magnificus, dear colleagues, friends and family

This inaugural address is devoted to food safety microbiology, the scientific discipline that studies pathogenic microorganisms that can be transmitted by food and the measures that can be taken to render food free from pathogens. However, to set the scene, I will first make a short excursion to a different domain where safety is also crucial for doing business: air travel, and show the remarkable progress aviation safety has made.



Figure 1. Airliner accident statistics. Source: Aviation Safety Network

Figure 1 shows the number of fatal accidents that happened worldwide involving civil aircraft with a minimum capacity of 14 passengers over the past seven decades. In the late fourties we see a peak of more than eighty accidents in one year, but especially since the beginning of this century the numbers decrease, and last year 'only' 29 accidents occurred. With 265 mortal victims 2013 was the safest year in aviation history since 1945. These numbers become even more impressive if we take into account that the volume of air traffic has increased drastically.

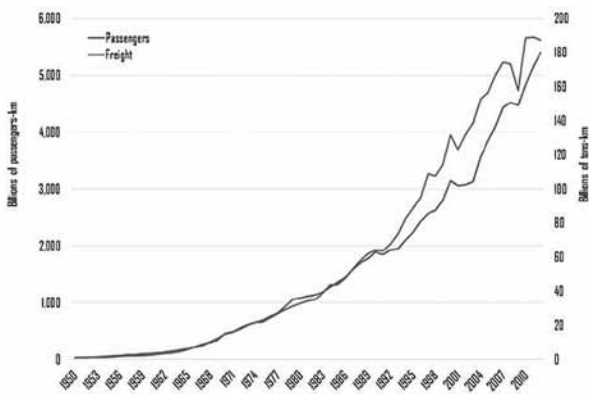


Figure 2. World air travel and world air freight carried, 1950-2012. Source: airlines for America

Figure 2 tells us that that the total distance travelled now is about ten times more than 40 years ago. Every accident is a tragedy, but the community of aviation safety experts can be proud of what they have achieved.

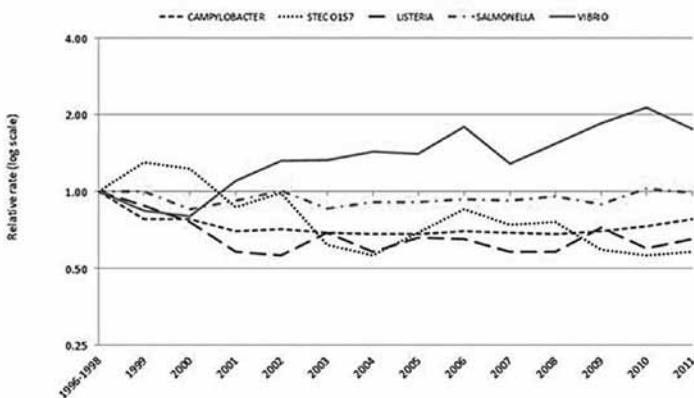


Figure 3. Trends in Foodborne Illness in the United States, 1996–2011. Source CDC

Estimating the prevalence of foodborne illness is much more difficult than counting airline accidents, and there is a large margin of uncertainty associated with the data shown here that reflects the situation in the US over a period of 15 years with respect to some of the most relevant foodborne pathogens: *Salmonella*, *Campylobacter*, *Listeria*, *STEC* and *Vibrio*. The number of US citizens has increased somewhat in the same period, and food intake and composition of the diet also have changed, but this cannot conceal that progress in food safety microbiology during at least this period is far less spectacular.

It is absolutely not fair to make this comparison, but I hope that I am not the only food safety microbiologist here that feels a bit uncomfortable, maybe jealous, when confronted with these figures. It is undeniable that great progress has been made with the development of measures to improve hygiene and with implementation of preservation techniques like fermentation, cooking, drying, refrigeration, etc. but much of this was achieved a long time ago, and the number of preventable foodborne outbreaks remains very high. What are we doing wrong? Is our science not good enough, or do we underestimate our opponents, the microorganisms? They merit a closer look.

The microbial world

Microorganisms are living creatures that are too tiny to be perceived by the naked eye. Because they are so small it is easy to be misled and underestimate their significance. Yet, for an outsider it should be obvious that this planet belongs to the microbes and that we, humans, are just a few late arriving guests. They may be inconspicuous, but they are extremely abundant and have an amazing metabolic diversity. There could be as many as 5×10^{30} microorganisms and together they represent almost half of the biomass on the earth. Without microorganisms higher forms of life (or should I say 'other' forms of life?) would not have developed. Microorganisms shaped the biosphere and play an essential role in the carbon and nitrogen cycle. Microorganisms are massively present in our gut and live on our skin, and we are only just starting to unravel the complexity of these ecosystems and appreciate the effect they have on our health.

However, from the perspective of a microorganism there is not a friendly relationship. Some appreciate us as a suitable substrate for growth, but most just tolerate or ignore us. The prokaryotes do not really need us. There are a few notable exceptions such as *Salmonella Typhi*, *Shigella* and *Variola* that are pathogens that depend on the human host to replicate. Most pathogens do not have such an exclusive taste and will also happily flourish in or on other animals (which is why they are referred to as zoonotic agents). It may well be that the extinction of mankind will also mean the end for a few strains that are exploited by us, such as *Lactococcus lactis* subspecies *lactis* biovar *diacetylactis* that is used for the production of fermented dairy products, but in general we are of little concern for them.

For us the relation with microorganisms is of utmost importance. We could not exist without them. Unfortunately, though, they are also our worst enemies. Just think of the dark middle ages when the bubonic plague was on tour, causing more than 100 million casualties, which inspired Pieter Bruegel to make this horrible but also fascinating painting.



Figure 4. *The Triumph of Death*. Pieter Bruegel the Elder in 1562. Museo del Prado, Madrid

More recently, at the beginning of the 20th century the Spanish flu took about 75 million lives which is almost 10 times more than the number of victims of the 'Great War'.

Last example, smallpox, a viral disease, killed more than 300 million people in the twentieth century alone. The eradication of this disease in 1977 should probably be regarded as one of the most, or maybe the most important achievement of science of all times.

But where do we stand today? To what extent are microorganisms still causing misery and death? The best answer for this comes from studies that are performed in the framework of the Global Burden of Disease project which is a collaborative effort between hundreds of experts from the World Health Organization (WHO), Harvard School of Public Health, the Institute for Health Metrics and Evaluation (IHME), and the World Bank. Instead of only using mortality as the measure of the impact of a disease or injury, the DALY concept is used.

The term 'DALY' stands for disability-adjusted life years, and also takes other adverse health effects into account. Depending on the severity of the effect a different weight is assigned, for example a common cold is a very mild disease, but the disability weight of kidney failure is more than 60%. Very arbitrary of course, but the DALY concept combines mortality and morbidity in one single metric, which greatly facilitates the comparison of the significance of different diseases and injuries.

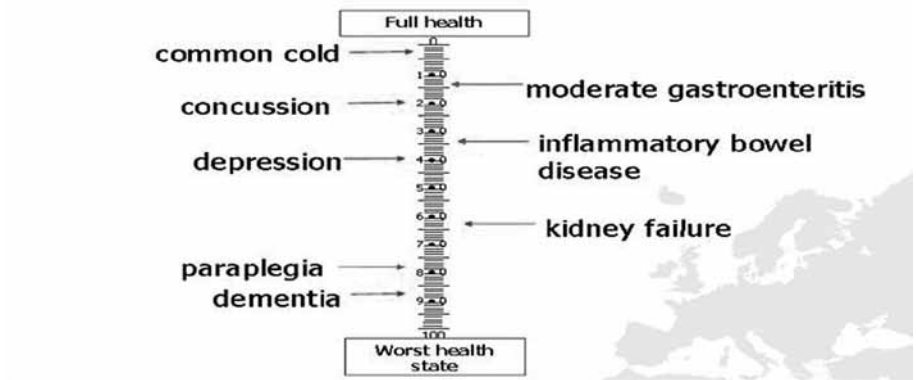


Figure 5. Disability weights for several diseases. Source: ECDC

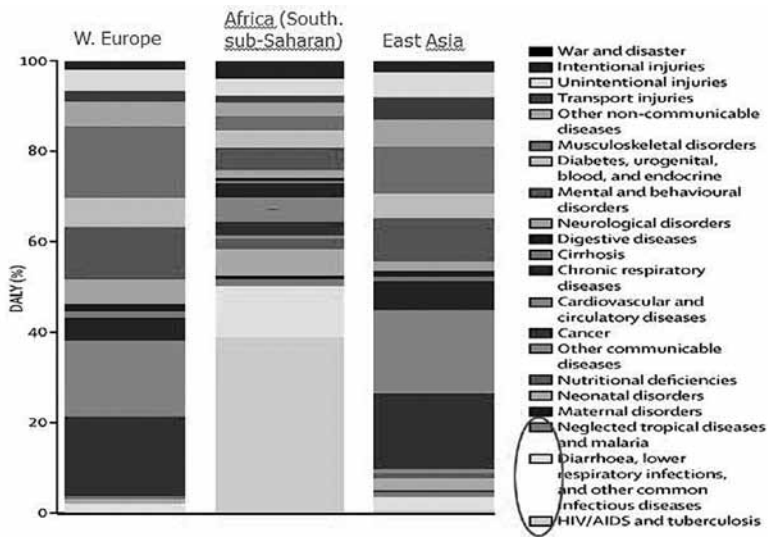


Figure 6. Percentage of DALYs by 21 main cause groupings and region. Global Burden of Disease study 2010. C.J. Murray et al. *The Lancet* (2012) 380:2197-2223

In figure 6 we can see the relative contribution of different diseases and injuries to the total DALY burden as a function of the geographical region as registered in 2010. The diseases and disorders have been grouped together. On the horizontal axis the different regions are indicated. The GBD project distinguishes 21 regions but for the sake of simplicity I have selected three: Western Europe, Southern sub-Saharan Africa

and East Asia. In Western Europe and in East Asia cancer, cardiovascular disease, but also musculoskeletal disorders and mental disorders are the main contributors to health loss. In these regions diseases caused by microorganisms account for just a few percent the total DALY burden. In less developed regions like Southern sub-Saharan Africa the role of microorganisms is much more important, in particular as causative agent of AIDS and of infant diarrhea which together account for more than 50% of the burden of disease.

If we want to further zoom in on the pathogenic microorganisms that are transmitted by food we need other information sources because the GBD project did not specifically address this. However, there is a very interesting study conducted in the Netherlands by the RIVM, the National Institute for Public Health and the Environment, that aimed at estimating the effect of the presence of pathogens in our food with the same DALY concept and comparing this with the effect of toxic chemicals and the impact of choosing unhealthy diets (too much fat and sugar, not enough vegetables and fruits to put it simple).

DALYs lost	Food			Other
	Microbiological	Chemical	Dietary	
>300,000			Unhealthy diet total	Cardiovascular diseases
100,000-300,000			Energy balance	Lung cancer
30,000-100,000			Too little vegetables, fruit, fish.	Alcohol consumption
10,000-30,000			Saturated fatty acids	Road traffic accidents
3,000-10,000	Gastroenteritis			
1,000-3,000				Bacterial meningitis
300-1,000	Campylobacter	Allergens Acrylamide		
<300	STEC 0157	PAHs		

Table 1. Health loss in DALYs per year caused by various factors. Source: C.F. van Kreijl et al. (2006) *Our food, our health*. RIVM, the Netherlands

The results have been published in a report entitled 'Our food our health' and show that the health loss due to microbiological contamination of food is in the order of magnitude of 3000-10000 DALYs per year. You may ask: how serious is it? This depends on how you look at it. Poor quality diets have a far greater impact of more than 300.000 DALYs, but when compared with chemical food safety which accounts for only 300-1000 DALYs annually if we exclude allergens, foodborne pathogens are more relevant, even though the public generally perceives chemical hazards in food as more threatening than microbial hazards.

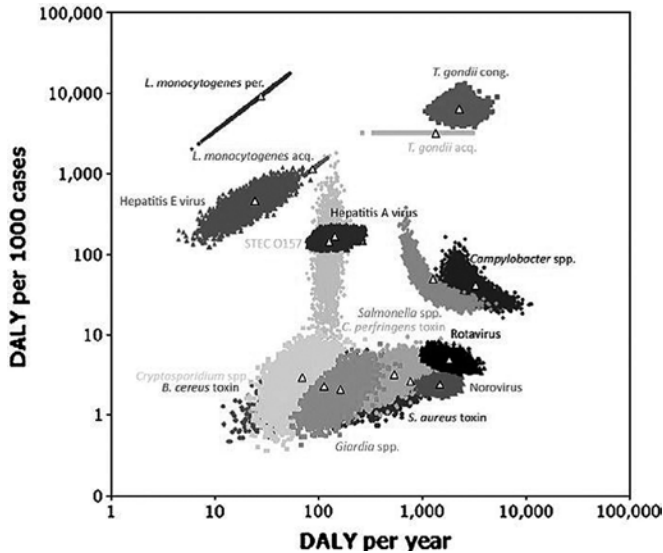


Figure 7. Ranking of pathogens by burden at population and individual level in the Netherlands, 2009
 Source: A.H. Havelaar et al. *Int. J. Food Microbiol.* 156 (2012) 231–238

The incidence of foodborne disease in the Netherlands (and other developed countries) is very high, but most foodborne infections are relatively mild which explains why their overall health impact on the population is quite limited. There are for instance more than one million cases of rotavirus and norovirus infections per year in the Netherlands, unpleasant indeed, but usually not very serious, accounting for only one- or two thousand DALYs per year. On the other side of this graph you can find *Listeria monocytogenes* with less than 100 cases per year. For those who are infected the consequences are often severe which is why a relatively low number of cases account for more than 50 DALYs.

It may be tempting to use the argument that foodborne illnesses are generally mild to downplay its relevance, but for food business operators there is always a compelling economic argument to take microbiological food safety very serious. Errors can be very costly indeed and undermine consumer trust. Recently Fonterra was confronted with a claim of over 300 million Euros from Danone, following the allegation that its whey powder was contaminated with *Clostridium botulinum*. It turned out to be a false alarm but the damage was already done with massive and expensive recalls and loss of reputation. Big companies have gone bankrupt as a result of foodborne outbreaks, and people have been sent to jail for neglecting food safety microbiology. In most cases food pathogens only cause a mild, self-limiting disease, but don't make the mistake to ignore them. Fortunately we *know* quite well what we have to do.

Prevention of foodborne outbreaks

In principle there are two possibilities to prevent foodborne outbreaks: either avoid exposure (ingestion) of pathogens or reduce the susceptibility to pathogens. The second option, however, is beyond the (direct) influence of food manufacturers; all they can and must do is making sure their products are not contaminated by one or a combination of control measures:

- Prevent or minimize contamination of the raw materials/ingredients
- Prevent or minimize growth during production and storage
- Apply a process that inactivates or physically removes pathogens
- Avoid recontamination

To illustrate what this means in practice you can think of plain cow's milk.



Figure 8. Album cover 'Safe as milk', Captain Beefheart, 1967. Buddha records

Back in the sixties some of us believed that milk is safe, but we know better now. Milk, in particular raw milk has a bad reputation as a transmission vehicle for pathogens such as *Salmonella*, *Mycobacterium bovis*, *Campylobacter*, STEC and *Brucella*. However, we can *make* it safe.

Prevention of contamination can be achieved by maintaining good agricultural practices, which includes ensuring that the animals are healthy, stables and milking facilities are clean and taking hygienic measures before and during milking.

To prevent growth the milk should be stored under refrigeration. However, some pathogens can grow, albeit slowly, at refrigeration temperatures, so the duration of the storage should be limited.

Inactivation can be achieved by pasteurization, a relatively mild heat process, typically 15 seconds at 72°C, that eliminates all the vegetative pathogens.

Prevention of recontamination can be achieved by filling the pasteurized product in a well-protected high hygiene zone and using adequate packaging materials that represents a physical hurdle.

For other food products we can apply the same principles. Quite simple, but until now nothing has been mentioned about microbiological analysis. When do we finally start testing? What is the role of testing in the context of food safety microbiology? The most important tests were actually already done before: microbiological analysis allowed us to identify the microorganisms that caused outbreaks, we investigated where they came from by testing samples from the farm environment, the factory, the processing equipment, packaging materials, etc, we used test methods to determine the effect of heat treatments on pathogens, and we identified potential recontamination routes based on the results of extensive testing of the factory environment.

Based on these results we know exactly how to manage microbiological hazards. However, if we are really sure why would we test finished products? Pasteurised milk is maybe not the best example because routine testing is usually limited to indicators, such as Enterobacteriaceae rather than individual pathogens, but the comment is equally valid for indicators: we know that the control measures are capable to eliminate them or reduce their number to an acceptable level so testing should not be necessary.

The problem is that we are not always so sure; for some products we do not have the evidence that the measures are truly effective, we may not fully trust that the measures were implemented as planned, or ingredient suppliers are not even willing to take the necessary control measures. Finished product testing should therefore be seen as an attempt to compensate for the lack of trust.

The presence of shiga-toxin producing *E. coli* on meat can serve as an example. This pathogen is quite common in the intestinal tract of healthy cows, and from there it is likely to contaminate raw meat during the slaughtering process. It can readily be inactivated by cooking or baking, but depending on the preference (or should I say ignorance) of the consumer the heat treatment may not be sufficient so we need to ensure that the raw meat does not contain this pathogen. Unfortunately the control measures that can be taken during slaughtering are not as effective as we would like them to be. Among other things there is a human factor: operators in slaughtering houses are under pressure to work fast, they don't get the training, nor the salary of let's say an airplane pilot, and if we may believe the findings that were revealed in a recent Dutch television program 'Zembla' more than half of the carcasses are contaminated with fecal material. 'Poepvlees' it was called, a term which I prefer not to translate. I can't judge if this is true, but it is understandable that meat producers do a lot of testing before they release their products, in particular in the US where rare burgers are not so rare and legislation is strict.

Indeed, end product testing may also be necessary to comply with legal requirements. I already mentioned the US, but Europe also has a strict legislation, for instance EC regulation number 2073 provides a long list of food safety criteria for various products. Food business operators are obliged to perform finished product testing as specified in the regulation.

The list includes the usual suspects like *Salmonella*, *Listeria*, *Campylobacter*, but you may find it surprising that the most dreadful pathogen, *Clostridium botulinum*, is not even mentioned. For a good reason though, testing for *C. botulinum* does not make sense. We have full confidence in the control measures. If we would really rely on testing, canned foods would not even exist.

These examples show that end product testing is mainly used if there is insufficient trust in the control measures, but to what extent can we restore trust with testing? This reflection leads to two other questions:

- How reliable are the methods we use?
- How many results do we need before we can draw a conclusion?

How reliable are microbiological test methods?

It's difficult to give a general answer on this question because there are so many different methods. We can distinguish methods that aim at enumeration, counting, of microorganisms, whereas other methods will only tell you if a microorganism is present or not. There are methods that are specific for one species, genus or family, and there are methods with a very broad scope like all culturable microorganisms,

or all yeasts and moulds. Methods also differ substantially in the technology used, which can vary from the good old petri dish to more sophisticated techniques such as RT-PCR or automated Elisa, and the performance or reliability is therefore also quite variable. However, to give you an idea I will explain the general principles of the detection method for *Salmonella*, which probably represents one of the most frequently used test methods in the food industry.

It all starts with a sample that was taken from a production batch, let's say 100 gram of milk powder. Even if there is only one single *Salmonella* present we want to be able to detect it. In theory we could make it visible under a microscope, but it's just not feasible to screen the entire sample by microscopic examination so we have to use tricks. A simple but very efficient trick is to make use of the desire of *Salmonella* to reproduce itself. If the conditions are suitable it can grow very rapidly, with a doubling time of less than 30 minutes. That means that the number of cells increases with a factor 4 (or more) in one hour, 16 in two hours, etc. So if we patiently wait 12 hours we'll have more than 16 million cells instead of just one. *Salmonella* is not very demanding, adding some water and leaving the suspension at a temperature of around 37°C is sufficiently seducing to make it grow and once it reaches a threshold of around ten or one hundred thousand cells per ml there are several possibilities to detect it such as using PCR, ELISA or selective plating methods.

Not every microorganism is so easy to manipulate; some require special growth media or growth conditions, others will not even multiply at all in artificial media so for these targets other tricks are required, but for *Salmonella* it's relatively easy to detect just one cell in a sample of 100 gram or more. For the chemists in this audience: one, presumably dehydrated, *Salmonella* in 100 gram corresponds with about 1 ppq (that's one part per quadrillion, or 10^{15}). In terms of sensitivity this longstanding method scores very well.

Sensitivity is an important aspect to judge the performance of analytical methods, but there are several other relevant criteria such as specificity and matrix compatibility.

- **Specificity:** relates to the extent to which a method is able to discriminate target from non-target microorganisms. If you think of a chemical analyte like bisphenol A or histamine the target is a well-defined molecule. Variability is usually limited to the presence of isotopes, or, for more complex molecules, to changes in the three dimensional configuration. For analytical microbiology the variability of the target is often huge. When we test for *Salmonella*, for example, we aim at a genus that comprises more than 4000 different serotypes. Even at the species level the genetic variability can be considerable, as shown in the next figure.

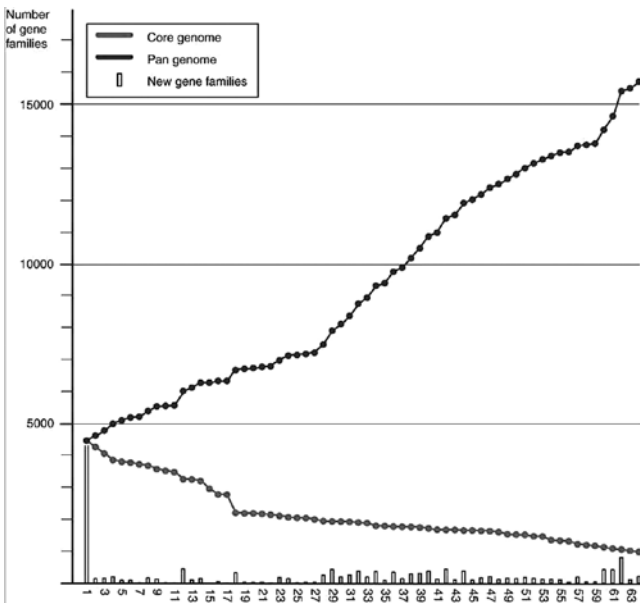


Figure 9. The core genome and pan genome of *E. coli*. Lukjancenko, O., Wassenaar, T.M., Ussery, D.W. (2010) Comparison of 61 Sequenced *Escherichia coli* Genomes. *Microb Ecol.* 60:708–720.

- Based on nucleotide sequence analysis of more than 50 strains of *E. coli* we know that the average *E. coli* genome size comprises about 5000 genes. However, from these 5000 genes less than 1500 are present in the genomes of all of the strains that were sequenced. The pan genome of *E.coli*, which is the total number of different genes or gene families that was found in the entire genome content of all the strains together, comprises more than 13000 genes. This study was done in 2010, and there is no doubt that when more sequences become available the size of the pan genome will further increase and that the size of the core genome will shrink. The conclusion remains the same though: what we call a single target comprises a wide variety of strains. We know very little about the impact of this genetic variability on the behavior during enrichment and isolation, the critical steps in detection methods, but it seems quite likely that depending on the genetic content some strains can be detected more easily than others. This is certainly something that needs further attention.

In some cases we even have to deal with moving targets, in particular when we aim at detection of newly recognized, or emerging pathogens, simply because the taxonomy lags behind. As an example you can think of *Cronobacter*, an opportunistic pathogen that is of particular concern for premature infants. The infection is very serious with a mortality rate of about 40%.



Figure 10. Francisco de Goya. Saturno (Cronos) devorando a su hijo. Museo del Prado, Madrid.

About twelve years ago several outbreaks occurred that were associated with contaminated infant formula, which spurred the manufacturers of these products and the authorities to develop and apply specific test methods. At that time the microorganism was called *Enterobacter sakazakii*, but it soon became clear that the boundaries of this species were not well defined. We found many strains that did not really fit well with the definitions, and it was quite impossible to decide if they were *E. sakazakii* or not, which obviously made it also impossible to design a specific method. Meanwhile, the taxonomy has been greatly improved, in large parts due to the efforts of Dr. Carol Iversen who meticulously gathered and characterized a large number of isolates that were thought to be *E. sakazakii* or close relatives. As a result of this work a new genus was proposed, *Cronobacter*, named after Cronos, one of the titans in Greek mythology, who devoured his own children immediately after birth. This genus now contains seven different species. At the same time new species were proposed for the innocuous *Cronobacter* lookalikes such as *Enterobacter pulveris* and *Enterobacter turicensis*. Now we know much better what we are looking for, and what is equally important, we also know what is outside the scope.

These were a few examples of the difficulties that we encounter to cope with the genetic variability of our target. On top of this there is also a physiological variability: factors like growth stage and environmental stress may drastically alter the behavior during enrichment. Damage to cells can for example increase the length of the lag phase, and thereby reduce the probability of detection. However, there is very

limited information on the magnitude of this problem, and we do not have specific solutions to improve the recovery of heat stressed cells or dry stressed cells.

Nevertheless, anticipating that such sublethally injured cells may be present we often use a non-selective environment for the first enrichment of our target, but this may lead to overgrowth or even inhibition by other microorganisms that we are not interested in. Other microorganisms that originate from the same sample can indeed represent a major problem, which leads to another important performance criterion:

- **Matrix compatibility:** obviously we prefer methods with which any type of sample can be analysed. However, in many cases we rely on the growth of the microorganisms to allow their detection and as the sample will form part of the growth medium, it must be verified that its chemical composition does not negatively affect the growth of the microorganism we are looking for. In general, however, this is not a problem. Most pathogens are quite tolerant with respect to their growth environment, and if *we* consider something suitable to eat it's probably also a good substrate for pathogenic microorganisms. There are a few notable exceptions though, like acidified foods or spices, but most of these problems can be overcome quite simply by adding a buffer or neutralizing agent in the diluent that we use to transform the sample into an enrichment medium.

- Of much greater concern is the presence of competitors. As long as a sample only contains the target microorganism, it is easy to detect, but most samples contain many other microorganisms and our analytical target could well be a small minority. The variability of the composition of this background microflora brings about much more uncertainty about the probability of successfully recovering pathogens from a sample than the variability of the chemical composition of foods. One can for example expect competition for nutrients, and we know that the metabolic activity of the background flora may result in production of organic acids that can suppress the growth of the target. There is also evidence of specific inhibition and growth promotion by the background flora, but we mostly do not know what the underlying mechanisms are, nor do we know which microorganisms behave as friends and which microorganisms behave as enemies. In other words, the non-selective enrichment is critical for the detection of microorganisms such as *Salmonella*, but it should be qualified as a black box.

In spite of these difficulties and challenges, there is a large amount of evidence from method evaluation and validation studies that confirm that microbiological methods perform very well with respect to sensitivity, specificity and matrix compatibility. It can be argued that such studies have limited significance because they are usually based on artificially contaminated samples prepared from relatively 'clean' matrices (in a microbiological sense, with a low background flora) and therefore not

necessarily sufficiently representative of what is really present in our food. However, we can also regard it from a more pragmatic perspective and ask the question if there is any evidence that outbreaks could have been prevented if only a better, more reliable, method would have been used to test products before release. For the sake of clarity: this question excludes outbreaks associated with products that were not tested at all, for example because a (specific) method was not even available, or because insufficient samples were tested. It also excludes products that were released in spite of a positive test result which unfortunately has happened more than once. Back to the question: have there been outbreaks that could have been prevented if only a better method would have been used? Well, *if* it has ever happened it must be extremely rare, I am not aware of any example, nor any of my colleagues who I consulted for this, which suggests that the occurrence of false negatives is of very low concern. The occurrence of false positive results on the other hand appears to be a bigger problem: the analytical result is positive but in reality pathogens are not present.

The screenshot shows the Canadian Food Inspection Agency (CFIA) website. The header includes the Government of Canada logo and navigation links for Canada.ca, Services, Departments, and Français. The main navigation bar lists 'About the CFIA', 'Food', 'Animals', 'Plants', and 'Guidance Document Repository'. A breadcrumb trail indicates the current page is 'Home > About the CFIA > Newsroom > Food Recall Warnings > Complete Listing > 2011-11-07'. A sidebar on the left contains a menu with 'Food Recall Warnings' selected. The main content area features a 'Notice' section with a disclaimer: 'This archive of previously issued food recalls and allergy alerts is provided for reference and research purposes. Users should note that the products listed in the archive have been subject to removal from the marketplace or appropriate corrective action. Food recalls or allergy alerts are not an indication of the food safety status of products produced at a later date.' Below this is a 'Consumer Advisory - Cancellation of Health Hazard Alert regarding Salmonella in Strubs ready-to-eat Danish Style Smoked Grav-Lox Atlantic Salmon'. A table provides details: Recall / advisory date: November 7, 2011; Reason for recall / advisory: Microbiological - Salmonella; Hazard classification: Class 2; Company / Firm: Foodfest International 2000 Inc.; Distribution: Ontario; Extent of the distribution: Retail. A 'Contents' link is visible at the bottom of the page.

Figure 11. Recall of smoked salmon based on false positive result. Source: Canadian Food Inspection Agency

False positive results

I already mentioned the whey product of Fonterra that did not contain *Clostridium botulinum*, but you can also think about the allegation that Spanish cucumbers were contaminated with STEC, pathogenic *E. coli*, in 2011. STEC was indeed present on

the cucumber, but it was not the virulent strain that was causing the big outbreak with its epicenter in Germany. More than 10 years ago a Swiss research group reported in respectable scientific journals that bottled water was contaminated with Noroviruses. Not just one bottle, more than 30 % of the tested samples were positive, representing many of the well-known international brands that you can find in all other European countries. False positives, not very unusual for an extremely sensitive technique like nested PCR, especially in the hands of an inexperienced student.

Finally an example which is of interest in relation to the large *Salmonella* outbreak that occurred last year in the Netherlands due to consumption of contaminated smoked salmon. In 2011, smoked salmon was recalled in Canada after a positive test result. It turned out to be a false positive result and the recall was cancelled. You may ask, why did the Canadians test for *Salmonella*? Did they know it is a significant hazard?

False positive results are annoying and can be very costly, but from a consumers-safety perspective they are not a real problem. For consumers finished product testing is only beneficial, but if we want to gain their trust based on finished product testing, how many tests should we perform?

How many results do we need before we can draw a conclusion?

An analytical result only tells us something about the sample we tested. Let's say we have analysed 25 gram of a product, and we did not find the pathogen we were looking for. We used a reliable method so we can be quite sure that the *sample* was not contaminated. However, that does not mean that the entire production batch is free from this pathogen. Unfortunately it is practically impossible to test the entire batch, it just would become too expensive, and besides, most of our methods are 'destructive', which means that nothing is left after testing and this is not going to change in the near future either. All we can do is to try to take a representative sample and hope that the conclusion for the sample is equally valid for the rest of the product. This is a reasonable assumption for products that can be homogenized and in which relatively large amounts of microorganisms are present. For instance, we have a liquid product, we stir it well, and find 100 or 1000 bacteria per milliliter. If we take several samples and if the resulting counts are consistently at this level we can draw a meaningful conclusion about the contamination level in the entire batch. However, many products cannot be mixed well. A commonly encountered practical constraint is the large size of production batches, but also because products are usually packed and distributed in relatively small units, such as bottles, cans or pouches and it cannot be assumed that pathogens are evenly distributed.

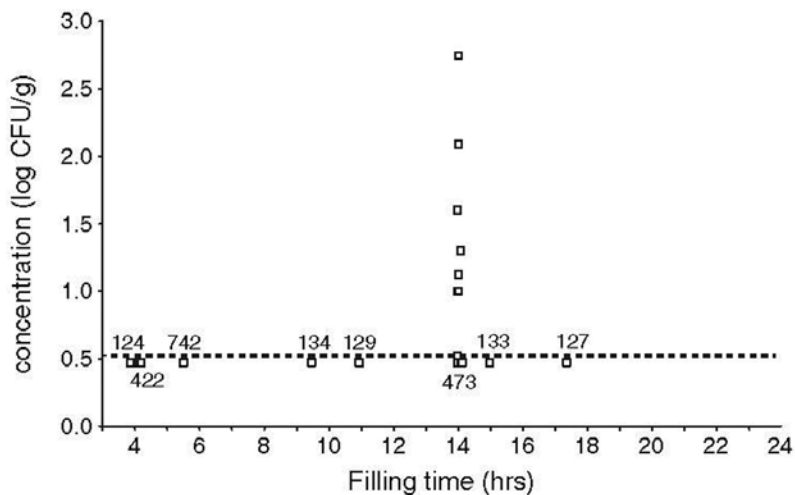


Figure 12. I. Jongenburger et al. *Int. J. Food Microbiol.* 151 (2011) 62–69

A very good example of how uneven pathogens can be distributed was given recently by Dr. Ida Jongenburger and coworkers here at Wageningen University. From a batch of powdered infant formula that was recalled because of the presence of *Cronobacter* she took 2290 samples of 1 gram and used a plate count method to determine the actual level of this pathogen in each of these samples. The results are presented in relation with the filling time and suggest that something went wrong after 14 hours. However, only 8 samples that came from cans that were filled at that moment gave a positive result, and the counts were very different from one sample to another, ranging from 3 cfu/g to about 600 cfu/g. 473 samples from cans that were filled at about the same time all gave negative results, just like the other 1800 samples from that batch. This example obtained with a naturally contaminated batch clearly illustrates the limits of testing. For this specific pathogen even very low levels are of concern, but if the distribution is so very inhomogeneous it is likely that we miss it unless we take hundreds or thousands of samples.

We can increase the probability of detecting a contaminated batch by taking larger size samples: instead of 1 gram we can analyse 10, 100 or even 1000 gram. Taking more samples and application of a continuous sampling procedure also increase the probability of detection. It is obvious that a higher overall contamination level will also make it easier to find a positive, so testing can certainly be of value to intercept heavily contaminated products, but low level contaminations are likely to escape from our attention.

Conclusion

This analysis can help us to define priorities for scientific research in this field. If improving food safety is the ultimate goal there is no strong incentive for developing better analytical methods for ingredients or finished products, unless they would be non-destructive and capable of screening an entire batch instead of just a few small samples, while maintaining or even improving the detection limit. That would be fantastic, but it will probably remain a fantasy for many decades, or more.

This does not mean that analytical microbiologists do not have a wish list. For instance, we would be very happy with a method that specifically detects *virulent* Shiga toxin producing *E. coli*, and for targets that do not replicate in vitro such as noroviruses it would be desirable if we were able to distinguish infectious from non-infectious particles. There can be other reasons to work on analytical methods, for example to get faster results, to make them more cost efficient, to provide validation data to gain international acceptance and facilitate trade, but it should be understood that this type of research will not directly make our products safer. For improving safety we should rather focus on designing smarter sampling plans, research that provides better insight in transmission routes, the ecology and behavior during food processing, research on new more efficient control measures, and microbiological risk assessment, which will also be the research priorities for the European chair in the coming years.

However, for real impact we also need to strengthen the safety culture. Root cause analysis often, very often, identifies human factors at the origin of a food borne outbreaks. In most cases the scientific knowledge on how to control hazards exists, but is not always used, either because of lack of training, or in other cases because food business operators do not wish to invest in safety and thereby put their customers at risk. In the beginning of this address I mentioned how much progress has been made in aviation safety. Probably there are other economic sectors that do better than us. I don't believe they have better science or better scientists, but when it comes to the safety culture the food industry as a whole still has a lot to learn.

Under the guidance of my predecessors Prof. Mike van Schothorst and Prof. Leon Gorris. the European Chair has made important scientific contributions to microbiological food safety ever since it was installed in 1997 However, the main reason for its existence is to provide education in Food Safety Microbiology , which is a pre-requisite for a healthy and strong safety culture. This task will remain a high priority for the Chair in the coming years, and we aim to further strengthen our impact with new education tools and by learning ourselves from other sectors for which safety is also paramount.

Credits

Reaching the end of this address I would also like to say a few words of thanks. There are many people to whom I am indebted for their support to me and to the European Chair:

In the first place I wish to thank Wageningen University, the members of the assessment committee and the executive board for granting me their confidence by appointing me to this position.

I am very grateful for the continuous support of and guidance by the industrial sponsors of the Chair, Mondelez, Danone, DSM, Unilever and Nestlé. I am honoured that several representatives of the sponsoring companies are present today and I look forward to a fruitful collaboration in the coming years.

Thank you to all my new colleagues in the Laboratory for Food Microbiology for helping me to get started and feel at home quickly. In particular to Prof. Marcel Zwietering for hosting the Chair but even more for sharing his insight, ideas and support.

Special thanks go to the members of the team that built the European Chair:

- my predecessors Prof. Mike van Schothorst and Prof. Leon Gorris for their invaluable efforts, not only on a scientific level but also for their contribution to the education program. I have very much appreciated your advice on how to find a balance between the expectations from the different stakeholders, the limited resources and my own ambitions.
- I would like to express my deepest and sincere gratitude to Dr. Martine Reij. With her experience and drive, Martine has played a pivotal role in the Chair for many years and without her help it would have been very difficult to take over. Martine, I sincerely hope that I can continue to count on you in the coming years.
- All the students who contributed to the scientific success of the chair. In particular I would like to thank the ex-PhD students Esther den Aantrekker, Chantal Kandhai, Els Biesta-Peters and Ida Jongenburger who I already mentioned. I also like to thank Lisa Gkogka who is in the process of finalizing her thesis and James Noah Ssemenda who recently started a Ph.D. project on risk assessment of fresh produce.
- Thanks Gerrieke van Middendorp for the enthusiasm with which you are working on MS based methods for identification of microorganisms and for giving support and advice to the master students.
- Many thanks also to Henri Dijkhoff who has been instrumental in setting up and continuously improving the digital learning courses. Distance learning is the future and I am very glad with your help to shape it.

Many thanks to my colleagues at the Nestlé Research Centre for acting as scientific sparring partner (even if I did not always give you much choice). The discussions, and disputes, on hot topics in food safety land, fundamental flaws of HACCP and a whole range of other, mostly microbiological, issues were always very inspiring.

Above all I wish to thank my friends and family, for being here today and on all those other moments when microbiology alone was not enough to keep me happy. I am sure that you are proud to see me standing here today, but this is not why I need you. You are so valuable because you make me realize that there are a few things that are more valuable than this funny outfit. Thanks for helping me to keep my feet on the ground.



Figure 13. *The Flying Dutchman*. Courtesy of J. Vangheluwe

To be honest, and as some of you know, sometimes I am not so firmly on the ground: occasionally I am flying recklessly just above the water, without any thoughts about aviation safety, on board of a Flying Dutchman (which is what this F and D stand for). But I am not flying alone....thanks co-pilots!

Finally, just in case my four most precious jewels have missed what I just said: muchas gracias niñas, por todo, for your love.

Ladies and gentlemen, thank you for your attention.

Ik heb gezegd.

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Prof. dr Han Joosten

Food producers conduct about 1 billion microbiological analysis per year, but the incidence of foodborne illness remains very high. Testing can be seen as an attempt to compensate for a lack of trust in the measures to keep pathogens under control during production. It is necessary to determine to what extent test results can restore trust. What are the scientific priorities? Develop more reliable methods, design better sampling plans, new antimicrobial agents or processing technologies, or should we rather strengthen the 'food safety culture'?