Survival of foodborne bacteria on wooden and plastic cutting boards

A laboratory protocol

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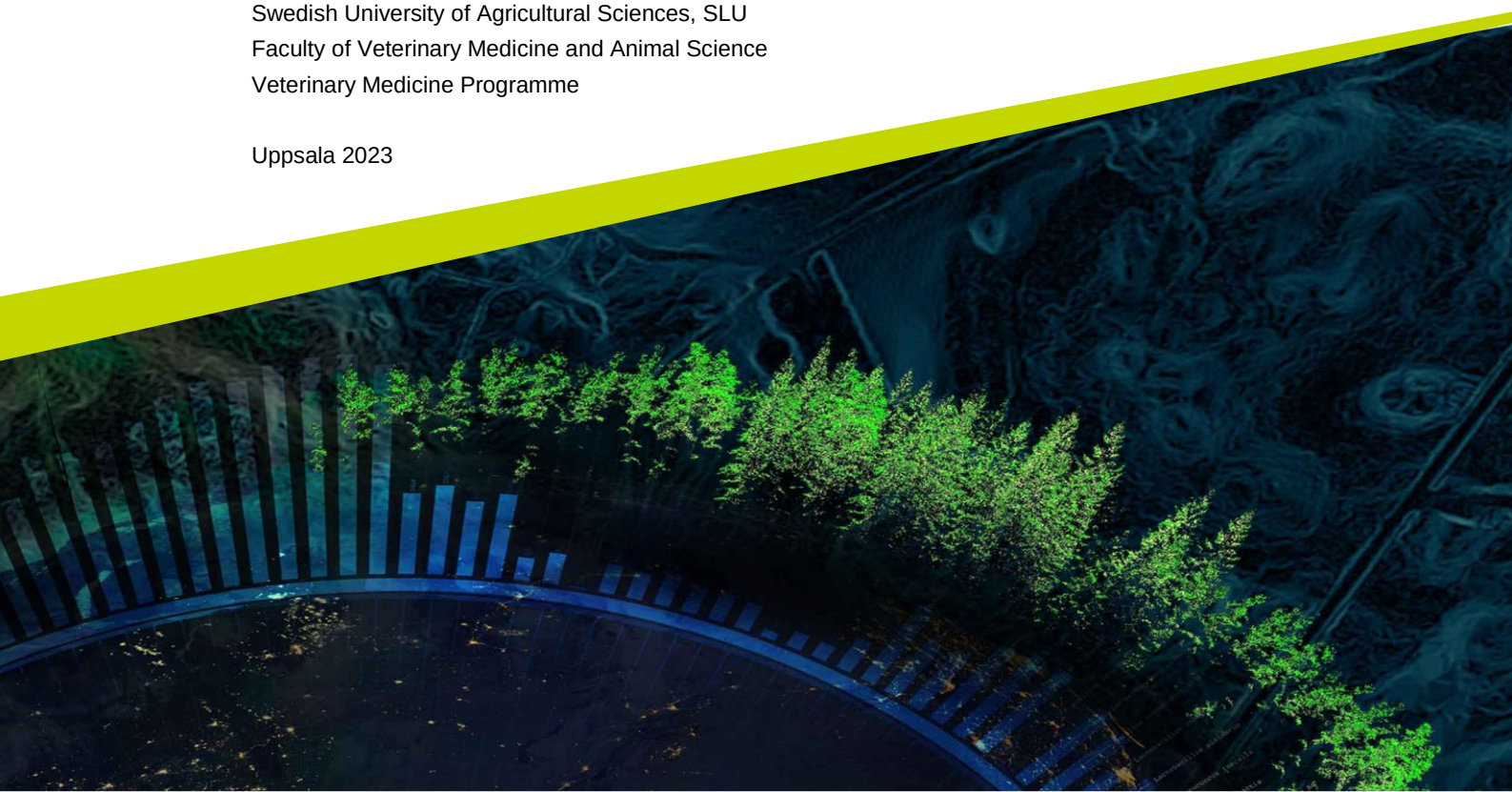

Survival of foodborne bacteria on wooden and plastic cutting boards - A laboratory protocol

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Abstract

The survival of foodborne bacteria on different cutting board materials has been studied and a functional laboratory protocol for further studies on the subject has been established. Six different bacteria, *E. coli*, *S. aureus*, *L monocytogenes*, *S. Typhimurium*, *Y. enterocolitica* and *C. jejuni* were inoculated on plastic (polypropylene) and wooden (bamboo and beech) cutting boards in concentrations 10^1 - 10^7 CFU. The samples were incubated for 24 hours and then either streaked directly on agar plates or incubated in enrichment medium before being plated. Growth or no growth for each bacterial species and each concentration was registered. Unexpected difficulties regarding contaminating background flora in the samples arose and were traced to the filtered chicken meat juice used when inoculating the bacteria and environmental contamination during the drying of washed cutting boards before inoculation. The results show that bacteria grow more readily on the surface of plastic cutting boards. The study also indicates a higher rate of survival on bamboo than beech. This was true for all tested bacterial species. Unfortunately, the results could not be validated, since time did not allow for enough replications of the experiments. A functional laboratory protocol is presented to be used in future studies on the subject.

Keywords foodborne bacteria, cutting board, bacterial survival, wood, plastic, laboratory protocol

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Abbreviations

ATCC	American Type Culture Collection
BCP	Bromocresol purple
BPW	Buffered peptone water
BHI	Brain heart infusion broth
CCUG	Culture Collection University of Gothenburg
CDC	Centre for Disease Control and Prevention
CFU	Colony forming unit
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
NB	Nutrition broth
OD600	Optical density at 600nm
SE	Staphylococcal enterotoxin
SFP	Staphylococcal food poisoning
SVA	National Veterinary Institute
WHO	World Health Organisation

1. Introduction

The World Health Organisation (WHO) estimated that around 600 million people globally fell ill from consuming contaminated food in 2010. Even though the WHO European region had the lowest estimated burden of disease still more than 23 million cases, and 5000 deaths was recorded. Out of these around 6 million cases of disease were due to foodborne bacteria (WHO 2017). In the EU the most common disease causing, zoonotic bacterial agents are *Campylobacter*, *Salmonella*, *Yersinia*, Shiga-toxin producing *E. coli* (STEC) and *Listeria*. Bacteria can also cause foodborne disease through their production of toxins, among these, *S. aureus* enterotoxins was the third most common cause in the EU in 2020 (EFSA & ECDC 2021a).

Contamination of food can occur during the entire processing chain, in slaughterhouses as well as in household settings. The most common place of exposure to causative agents is the household setting. In household settings routes of contamination can be food handlers, cross-contamination via kitchen utensils, inadequate heat treatment of food, storage abuse or inadequate chilling. Cutting boards, both plastic and wooden are well used kitchen utensils in domestic settings. If you were to use a cutting board to chop chicken one day, forget to wash the board and leave it on your kitchen bench until the next day. Would there be a possibility of cross-contamination even after 24 hours, and would there be a difference in risk between wooden- and plastic cutting boards? This study aims to present a functional laboratory protocol that could be used to try to answer these questions.

2. Literature Review

2.1 Foodborne bacteria

2.1.1 *Salmonella* spp.

Characteristics

Salmonella belongs to the family *Enterobacteriaceae*. The genus *Salmonella* is divided into two species, *Salmonella enterica* and *Salmonella bongori*. Under the species-name *S. enterica* 6 different subspecies are classified, each named and designated a roman numeral. A distinction of serotypes within the different subspecies can be made on the basis of somatic, flagellar and capsular antigens (Adams *et al.* 2016). There are over 2,600 know serotypes as of today (Graziani *et al.* 2017). Most serotypes that are pathogenic to humans are of zoonotic origin with the exception of *S.* Typhi and *S.* Paratyphi A, B and C which are host specific to humans and cause the systemic and sometimes lethal disease, enteric fever (Adams *et al.* 2016). The serovars *S.* Enteritidis and *S.* Typhimurium are the most common causes of gastrointestinal disease due to *Salmonella* in the EU (EFSA & ECDC 2021a).

Most of the *Salmonella* serovars has a recorded growth between 5-47˚C but with an optimum at 37˚C and they are killed at temperatures >70˚C. They have a wide pH range (4-9) at which they can grow and though they do require high water activity to grow they can survive in dry food with low water activity (Graziani *et al.* 2017).

Salmonella in foodstuff

Salmonella can be found in a variety of foods, eggs, meat, poultry, milk and vegetables. Chocolate and ready-to-eat food have also been a cause of salmonella outbreaks. Vegetables can be contaminated both during preharvest and postharvest steps in the process chain through for example salmonella containing animal manure used as fertilizers or contaminated irrigation water (Heaton & Jones 2008). A study showed that *S.* Typhimurium could survive for several weeks after contamination of carrots and radishes through inoculated irrigation water (Islam *et*

al. 2004). *Salmonella* can be persistent in a flock or herd of animals due to its longevity in the environment. Both infected and asymptomatic animals can shed bacteria through their faeces and in turn contaminate their living-environment, feed and even irrigation water – these can then be contamination sources for new animals in the group (Doyle *et al.* 2019).

In Sweden a severe outbreak of *S.* Typhimurium in 1953 prompted the start of the *Salmonella* control programme to prevent *Salmonella* in all parts of the production chain. Today Sweden has a very low incidence of domestic *Salmonella* cases. The animal surveillance part of the Swedish Salmonella control programme consists of a voluntary and a compulsory part and includes both poultry, pigs and cow herds. The compulsory programme for poultry is meant to assure that poultry sent to slaughter are free from *Salmonella*. All poultry flocks with more than 500 birds must be tested and so do all holdings that sell egg for consumption and all breeding flocks with more than 250 birds. The voluntary programme for poultry is meant to prevent introduction of *Salmonella* to the heard and minimize the risk of spread to other animals and humans. In 2020 no *Salmonella* was detected in any of the 4,147 samples taken. No positive *Salmonella* samples were found on any of the 2,792 neck skin samples from broilers after slaughter (SVA 2020). In contrast Spain, Italy and Slovakia had positive tests reaching 16.9%, 24.6% and 40.3% respectively (ESEA & ECDC 2021a).

The compulsory programme for cattle and pig herds aim to ensure a low prevalence of *Salmonella* in domestic herds. Samples are also taken of pig and cattle carcases and lymph nodes at slaughterhouses and cutting plants. The prevalence varied from 0.03% in cattle carcases to 0.43% in breeding pig lymph nodes.

Salmonellosis

Salmonellosis was the second most common foodborne gastrointestinal infection in the EU 2020 (EFSA & ECDC 2021a). A seasonal trend has been seen 2011-2020 with more case reports during the summer months. In Sweden the number of annually reported cases has been between 1993 and 2279 during the years 2016-2019. Since the COVID-19 pandemic the number of reported cases of *Salmonella* has dropped to below 1,000 cases annually (Folkhälsomyndigheten 2021).

The infectious dose is estimated to be around 10^3 to 10^4 CFU, outbreak modelling studies have suggested an infection dose as low as 10 to 36 CFU depending on the conditions of the outbreak and the susceptibility of the host (Doyle *et al.* 2019).

The transmission occurs via faecal-oral route either directly from an animal reservoir or indirectly through contaminated food or environment (Graziani *et al.* 2017). According to a retrospective study of *Salmonella*-cases in the US the median incubation time was usually between 12-96 hours (Eikmeier *et al.* 2018). Symptoms include acute onset of vomiting, fever, diarrhoea (sometimes bloody), abdominal

cramps, myalgia and headache. Usually, the disease is self-limiting but serious complications can occur especially in young or immunocompromised people (Dhanoa & Fatt 2009).

2.1.2 *Listeria monocytogenes*

Characteristics

Listeria monocytogenes and *Listeria ivanovii* are the only two known species of *Listeria* pathogenic to humans. *L. monocytogenes* is ubiquitous in the environment and can survive during harsh conditions. It is able to colonize within food processing facilities and can be found in areas that are hard to clean such as drains and cracked surfaces (Silva *et al.* 2008; Overney *et al.* 2017).

According to the literature, the optimum growth temperature for *L. monocytogenes* ranges from 30-37°C. It can maintain growth between 0-45°C, but the growth will slow markedly at lower temperature. Heat-resistance is relatively low, *L. monocytogenes* is inactivated at temperatures above 50°C, a few minutes at 60°C and a couple of seconds in 70°C. The range of pH-value at which *L. monocytogenes* can grow is wide, from 5.5-9.6, but it has been shown to initiate growth in laboratory medium at a pH-value of 4.4. *L monocytogenes* is also relatively tolerant to high salt concentrations and can grow at a NaCl concentration of 10% but can survive concentrations as high as 20-30%. The minimum water activity for growth is 0.93 with an optimum at 0.97 (Adams *et al.* 2016; Rees *et al.* 2017; Doyle *et al.* 2019).

Listeria in foodstuff

Listeria monocytogenes can be found in many different food products. The source of contamination can be both the food processing environment and raw material used during food processing (Wiedmann 2002).

RTE-food of animal origin such as meat products, dairy and seafood products are readily associated with outbreaks of *L. monocytogenes* (EFSA & ECDC 2018a; EFSA & ECDC 2019). These type of high-risk food products are usually processed during low temperatures and with no further heat-treatment before being sold to the consumer which can favour the growth of *L. monocytogenes*. Frozen food products have also been associated with listeriosis, ice cream and frozen vegetables have been sources of outbreaks in the US (CDC 2022) and in the EU an outbreak due to contaminated frozen corn (EFSA & ECDC 2018b)

L. monocytogenes does not change the odour, taste or appearance of the contaminated product and can therefore be hard to detect for the consumer.

Listeriosis

L. monocytogenes is the main pathogen when it comes to cases of human and animal listeriosis. *L. ivanovii* is primarily pathogenic to animals, predominantly ruminants (Gouin *et al.* 1994; Dunnett *et al.* 2020). Only a few sporadic cases of *L. ivanovii* have been reported in human patients with underlying immunosuppressive conditions (Cummins *et al.* 1994; Guillet *et al.* 2010).

The lethality rate of listeriosis is high, the years 2017, 2018 and 2019 the overall case fatality rates were 13.6%, 15.6% and 17.6% respectively in the EU (EFSA & ECDC 2021b). The number of reported cases is low, a total of 1878 in 2020 and the overall trend for cases between 2016 and 2020 is steady with no significant increase or decrease. Infection with *L. monocytogenes* were most common at ages above 64 years (EFSA & ECDC 2021a). A recent study (Hafner *et al.* 2021) has shown a prevalence of *L. monocytogenes* in faeces of asymptomatic humans to be 10% while in earlier studies the prevalence has been 1-5% (Hof 2001; Grif *et al.* 2003).

There are several syndromes associated with *L. monocytogenes* that can be either invasive or non-invasive. Non-invasive infection leads to a febrile gastroenteritis which is a relatively mild disease; often self-limiting, that infect healthy people, usually due to high intake of bacteria through contaminated food. Clinical symptoms for febrile gastroenteritis caused by *L. monocytogenes* include headache, fever, diarrhoea and myalgia. Invasive infection can lead to more serious syndromes like neonatal meningitis, hepatitis and sepsis in infants and adults (Schlech 2019). Pregnant women, neonates, elderly people and immunocompromised persons are at a higher risk of acquiring *L. monocytogenes* infection (Goulet *et al.* 2012).

The incubation period for the disease seem to vary depending on the clinical presentation, a study done by Goulet *et al.* (2013) showed a median incubation time of 24 hours for gastroenteritis linked to *L. monocytogenes* while the incubation time for pregnancy-associated cases linked to *L. monocytogenes* showed a median of 27.5 days.

2.1.3 *Campylobacter* spp.

Characteristics

The genus *Campylobacter* belongs to the family *Campylobacteraceae* and in the year of 2022, includes 43 different species (Parte 2020a). *Campylobacter* has been known as a problem in veterinary medicine since the early 20th century as a cause of abortion in sheep and cattle. It was not until the 1970s and the development of selective media that the species *Campylobacter jejuni* and *Campylobacter coli* was noticed as major pathogens among patients with diarrhoeal disease (Skirrow 1977).

The alimentary tract of wild and domestica animals such as wild birds, poultry, cattle, sheep, dogs and cats is the principal environmental reservoir for *Campylobacter*.

Campylobacters are generally sensitive to environmental factors. They are microaerophilic and thrive in an atmosphere containing higher carbon dioxide (5-10%) than oxygen (3-5%). All species of *Campylobacter* grow at 37°C; with an optimum temperature for *C. jejuni* and *C. coli* at 41-45C. They do not grow below 30C (Adams *et al.* 2016; Doyle *et al.* 2019).

Survivability in room temperature is very low, but they can survive in low numbers in chilled milk and water (4°C) and frozen poultry products for several weeks. *Campylobacters* are sensitive to heat and are inactivated by pasteurization and boiling temperatures, they are also sensitive to drying and reduced pH (Adams *et al.* 2016; Doyle *et al.* 2019).

To evade various stresses (starvation, osmotic stress, extreme temperatures and chemicals) *Campylobacter* can enter a viable but non-culturable state where they are still infective but cannot be plated on routine culture media (Oliver 2005).

Campylobacter in foodstuff

Meat from broiler chickens and raw milk has been the leading cause of campylobacter-related foodborne outbreaks the past years (EFSA & ECDC 2021a). Because of the low infectious dose and the possibility of *Campylobacter* to survive in low temperatures, undercooked or deliberately non-heated products can be a source of infection. Cross-contamination from contaminated meat via kitchen utensils to non-contaminated food can also be a route for contracting the bacteria (Guyard-Nicodème *et al.* 2013).

During slaughter and evisceration carcasses can easily be contaminated with *Campylobacter* since it commonly occurs in the gastrointestinal tract of domestic animals and birds. Chilling the carcasses usually lowers the count of viable bacteria significantly due to the dehydration that takes place during this process. One study showed a decrease form 56.7% positive samples before chilling to 1.7% positive samples after (Nesbakken *et al.* 2008). The small size of poultry carcasses giving less of a drying effect when chilled and the unique surface texture of poultry skin probably contributes to enhanced survival of *Campylobacter* in poultry meat (Adams *et al.* 2016). Characteristic for *Campylobacter* outbreaks are a high number of sporadic cases rather than single-source outbreaks.

In Sweden, a voluntary control programme for *Campylobacter* initiated by the organisation Svensk Fågel has existed since 1991. The programme includes around 99% of all the broilers slaughtered in Sweden. The aim is to keep the annual prevalence below 10%. Ten individual caecum samples from each slaughter group are collected and analysed for *Campylobacter*. In 2020 and 2021 the prevalence of campylobacter has been 5.1% and 5.7% respectively (SVA 2020; 2021). In EU the

prevalence of *Campylobacter* in broilers varies but in 2020 it was 24.5% (EFSA & ECDC 2021a).

Campylobacteriosis

Campylobacteriosis has been the most reported foodborne gastrointestinal disease in the EU since 2005. Due to the withdrawal of the United Kingdom from the EU and the Covid-19 pandemic the number of cases has dropped markedly during 2020, totalling 120,946 cases. This represents a decrease in the *Campylobacter* notification rate of 25.4% since 2019 (EFSA & ECDC 2021a).

C. jejuni have a very low infectious dose, a dose of 500 bacteria can lead to disease in experimental human infections (Robinson 1981). The symptoms can vary from mild to severe. The most common symptoms are related to the gastrointestinal system with acute diarrhoea and abdominal pain, fever and headache. The symptoms usually occur 24 to 72h following infection and gradually resolve during a time period of up to a week (Blaser 1997).

Complications following an infection with *Campylobacter* can include irritable bowel syndrome, reactive arthritis or neurological syndromes such as Guillain-Barré syndrome (Allos 1997; Pope *et al.* 2007; Berumen *et al.* 2021).

2.1.4 *Escherichia coli*

Characteristics

Escherichia coli is a bacterial species belonging to the genus *Escherichia* and to the family *Enterobacteriaceae*. *E. coli* is the type species for the genus *Escherichia* and is also one of the most well studied bacterial species in history.

E. coli is a commensal bacteria in the gastrointestinal tract of humans and other warm-blooded animals, many of the strains are non-pathogenic but there are also pathogenic strains that can cause a variety of disease in both humans and animals (Doyle *et al.* 2019). Intestinal pathogenic *E. coli* (IPEC) are obligate pathogens which cause gastrointestinal disease while extraintestinal pathogenic *E. coli* (ExPEC) are facultative pathogens which live as commensals as a part of the normal gut flora in a fraction of the healthy population (Köhler & Dobrindt 2011). IPEC are further categorized into pathotypes (Table 1.) based on their different virulence determinants which control adhesion, invasion, motility, toxins, antiphagocytic surface structures and genetic characteristics (Jesser & Levy 2020).

E. coli has a high tolerance for environmental stresses. The bacteria can grow from 7-10°C up to 50°C with an optimum at 37°C (Adams *et al.* 2016). It can grow at pH 4.0-4.5 and at water activity of 0.95. A study showed that *E. coli* survived fermentation, drying and storage for up to 2 months at 4°C if inoculated at high numbers in fermented sausages (Glass *et al.* 1992). *E. coli* heat resistance is 45

seconds at 60°C, pasteurization and proper heating of food are important steps to inactivate the bacteria (Doyle *et al.* 2019) .

Escherichia coli in foodstuff

STEC is the pathotype most commonly linked to foodborne outbreaks both in Europe and in the USA (Adams *et al.* 2016). In Europe, the STEC/VTEC pathotype has been associated with foodborne outbreaks of haemoragic colitis and haemolytic uremic syndrome since 1982, particularly the serotype EHEC O517:H7 (Adams *et al.* 2016; Socialstyrelsen 2014). Outbreaks have been linked to sources such as meat of cattle origin not properly cooked, drinking water and fresh produce.

Factors associated with foodborne illness caused by STEC/VTEC are under-cooked or raw meat and meat products, for example undercooked hamburgers and cold smoked sausages. In slaughter houses the carcasses can be contaminated during evisceration and dehiding process, but also by cross-contamination due to contact with other carcasses or through contaminated equipment (Grispoldi *et al.* 2020). Samples of bovine meat taken at slaughterhouses in the EU 2016-2019 showed that 2.1% of the meat were contaminated with STEC/VTEC and for pig meat the same was true for 2.0% of the meat (EFSA & ECDC 2021a).

Other vectors are unpasteurized milk and contamination of vegetables during cultivation through watering with contaminated water or fertilizing with contaminated manure. It is also possible to contaminate ready-to-eat food through cross contamination or improper hygiene by the one handling the food (Socialstyrelsen 2014).

Disease associated with Escherichia coli

In 2020 STEC/VTEC was the fourth most common cause of foodborne illness in the EU with 4446 reported cases. A drop in the number of cases and in the notification rate in EU from 2019 to 2020 was seen. The decrease in notification rate were 22.4% and 18.2% compared with the rate in 2019. This drop is likely linked to the COVID-19 pandemic. In the EU, STEC/VTEC is the only pathogenic *E. coli* under surveillance (EFSA & ECDC 2021a).

The infectious dose for EHEC is very low, possibly lower than 100 organisms. A study from a large outbreak of 0517:H7 in western USA showed a suggestive infectious dose of less than 700 organisms (Tuttle *et al.* 1999). Another study showed that the infectious dose when consuming dry, fermented salami ranged between 2-45 organisms (Tilden *et al.* 1996). The capability of EHEC for person-to-person and waterborne transmission is also suggestive of a low infectious dose (Doyle *et al.* 2019). The 0517:H7 strain also shows a 30% risk of developing HUS and a case fatality of 11%. The diseases caused by the different *E. coli* pathotypes is listed in below (Table 1).

Table 1. List of Intestinal pathogenic E. coli *pathotypes and the disease they cause in humans.*

Pathotype	**Abbreviation**	**Disease**
Enterotoxigenic *E. coli*	ETEC	Infantile diarrhoea in developing countries, traveller's diarrhoea
Enteropathogenic *E. coli*	EPEC	Infantile diarrhoea in developing countries, traveller's diarrhoea
Shiga-toxin producing *E. coli*	STEC/VTEC	Shigella-like dysentery, hemolytic uremic syndrome (HUS), trombotic trombocytopaenic purpura
Enteroinvasive *E. coli*	EIEC	Shigella-like dysentery, mostly in developing countries
Enteroaggregative *E. coli*	EAEC	Acute and persistent diarrhoea in children in developing countries and travellers
Diffusely adherent E. coli	DAEC	Childhood diarrhoea

This table is an adaptation of Adams et al. *2016.*

2.1.5 *Yersinia enterocolitica*

Characteristics

Yersinia belongs to the family *Enterobacteriaceae* and contains three species which are pathogenic to humans, *Yersinia enterocolitica*, *Yersinia pestis* and *Yersinia pseudotuberculosis* (Doyle *et al.* 2019). *Y. enterocolitica* and *Y. pseudotuberculosis* can both cause foodborne illness, with *Y. enterocolitica* being the major causative species.

As a psychotropic bacteria *Y. enterocolitica* is able to grow from -1°C to +40°C but its optimum being around 29°C. The optimal pH for growth ranges between 7-9 (Adams *et al.* 2016) with a minimum of 4.2 ± 0.2 at 22°C (Bari *et al.* 2011).

Y. enterocolitica is sensitive to heat though the sensitivity can vary between strains. In one study, measured D-values for *Y. enterocolitica* in milk varied between 0.24-0.96 minutes at a temperature of 62.8°C (Lovett *et al.* 1982). The D-value is defined as the time (in minutes) of exposure at a given temperature that causes a one-log or 90 per cent reduction in the population of a specific microorganism. A later study on thermal inactivation of *Y. enterocolitica* through scalding in slaughterhouses showed a D-value of 2.5 minutes at 60°C and estimated a D-value of 0.6 minutes at 65°C (Bolton *et al.* 2013).

Yersinia in foodstuff

Pigs have been shown to be reservoirs for human pathogenic *Y. enterocolitica*. The most common bioserotype involved in human Yersiniosis is 4/0:3, this bioserotype is also commonly isolated from the pig population. Other animals have also been shown to be reservoirs for *Y. enterocolitica*, but these strains have mostly been of variants non-pathogenic to humans except for some sporadic cases (Socialstyrelsen 2013).

A study made in Finland showed that 60% of pig tonsils from fattening pigs at slaughterhouses were positive for *Y. enterocolitica*. The prevalence was higher in pigs that came from fattening-farms than farrowing-and-fattening farms (Rahikainen Ibañez *et al.* 2016).

Yersinosis

In 2020 the annual report from ECDC showed that Yersiniosis was the third most common cause of foodborne illness in the EU/EAA with 5668 cases of illness reported. *Y. enterocolitica* was the primary causative species followed by *Y. pseudotuberculosis* (ECDC 2022). An infective dose of *Y. enterocolitica* has not been concluded in studies but an estimated minimum dose of 10^4–10^6 has been suggested by FDA (FDA 2012).

The clinical presentation of Yersiniosis can vary with age. In older children and adults, it may include fever and appendicitis-like pain in the abdomen. In young children, diarrhoea – often bloody and fever can occur. The symptoms usually lasts 1 to 3 weeks but in rare cases they have lasted for weeks to months (Ostroff *et al.* 1992).

2.1.6 *Staphylococcus aureus*

Characteristics

The genus *Staphylococcus* is a member of the family *Micrococcaceae*. There are currently 63 known species of *Staphylococcus* (Parte 2020b). *Staphylococcus aureus* is the one principally associated with food poisoning in humans through its production of staphylococcal enterotoxins (SEs). A novel species, *Staphylococcus argenteus* was recently distinguished from S. aureus (Holt *et al.* 2011; Tong *et al.* 2015) and has been identified as a cause of staphylococcal food poisoning (SFP) outbreaks in Tokyo (Suzuki *et al.* 2017).

The first toxin isolated, SEA was discovered in 1959 and today around 25 SEs are known (SEA–SE*l*Z). They are separated into staphylococcal enterotoxins (SEs) and staphylococcal enterotoxin-like toxins (SE*l*) by whether or not they provoke an emetic response. The SEs are produced by *S. aureus* during growth and are very resilient to external stressors. The SEs work through stimulation of the vagus nerve and thus evoking an emetic response. Some SEs can also cause diarrhea (Etter *et al.* 2020).

Staphylococcus aureus can grow between 6-48˚C with an optimum of 35-41˚C but the temperature range of enterotoxin production is slightly narrower. The pH optimum lays at 6-7 but it can survive in the range of 4-10. *S aureus* can grow in a water activity of 0.86, while most competitive bacteria is inhibited at this value (Doyle *et al.* 2019). It has a relatively high but varying degree of heat resistance. It is also worth noting that *S. aureus* has a high tolerance for growing in media containing high concentrations of salt and it grows steadily at a concentration of 5-7% NaCl (Adams *et al.* 2016). Some strains can even grow at 20% NaCl (Nunheimer & Fabian 1940).

Staphylococcus in foodstuff

Staphylococcus aureus is naturally occurring on skin and mucous membranes in humans and animals. It can therefore contaminate food via human hands during handling and processing. A good hand hygiene in the kitchen is therefore important to lower the risk of *S. aureus* growing in food. *S. aureus* can be isolated from raw meat and poultry as a component of the skin microflora and can also be isolated from raw milk. This is usually not a problem since *S. aureus* is a poor competitor and is eliminated by pasteurization and cooking. However, the enterotoxins produced by the bacteria are heat-stable and outbreaks linked to heat-treated dried milk and chocolate milk has occurred (Adams *et al.* 2016).

In one outbreak, at a hotel in Germany, freshly produced ice-cream was the cause. Thirteen out of 31 guests reported symptoms of illness. Though it was not possible to find a common source of the contamination it was most likely linked to contaminated ingredients or equipment used for the production (Fetsch *et al.* 2014).

Staphylococcal intoxication

Enterotoxins formed by *S. aureus* was the third most common reason for food poisoning due to intoxication in Europe year 2020, with 402 cases reported (EFSA & ECDC 2021a). Due to SPF being a self-limiting and relatively mild gastro-intestinal disease it is highly possible that the reported number of cases far under-estimate the actual number of cases.

The onset of symptoms is rapid, usually between 1-6 hours and the symptoms often include vomiting, abdominal cramps and diarrhoea. Recovery from symptoms happens after a few hours up to one or two days (Stewart 2017).

2.2 Cutting boards

2.2.1 Studies on cross-contamination and bacterial survival

Cross-contamination of foods via cutting boards and other kitchen utensils have long been a known risk of foodborne illness. Wood has been the traditional material used for cutting boards, but in the early 1970s when various polymers became available, plastic cutting boards entered the market. Several studies have been done to analyse the risk of cross-contamination via kitchen utensils and the survivability of the bacteria. A study from 1993 showed a markedly less recovery of bacteria from the surface of wooden cutting boards compared to plastic cutting boards. In this study experimental contamination with monoculture of *E. coli*, *L. monocyto-genes* or *S.* Typhimurium was performed on used and new plastic and wooden cutting boards. Sampling of contamination in this study was done 0 and 3 minutes or 10 minutes and 12-18 hours after inoculation (Ak *et al.* 1994).

In a study by Schönwälder *et al.* (2002), it was shown that at higher bacterial concentration of *E. coli* (10^6 CFU/cm^2) the concentrations decreased faster on wooden surfaces than on plastic ones. However, at lower bacterial concentrations (10^3-10^4 CFU/cm^2) the materials behaved similarly and after a short time interval the bacterial concentration was reduced to none or very few on both plastic and wooden cutting boards.

In the same study (Schönwälder *et al.* 2002), they also compared bacterial reduction between different types of wood (unwashed and washed pine-heartwood, beech and poplar) and showed that bacterial translocation to the interior of the wood happens. They also showed that pine-heartwood had an antibacterial effect compared to the other types of wood. In the experiment the wooden blocks were placed in *E. coli* suspension resulting in an average germ load per block of 3-5 x 10^6 CFU/cm^2. After 24 hours the concentration on the surface of poplar and beech wood was still very high with only a low reduction at 3mm depth. For both the washed and unwashed pine-heartwood no *E. coli* could be recovered at depth after 3 and 5 hours respectively.

3. Material and Methods

3.1 Laboratory protocol

3.1.1 Preparation of bacterial cultures

This study included six different common foodborne bacterial species (Table 2). All strains except the *C. jejuni* strain were kept as reference strains at SVA and were re-plated on blood-agar plates and incubated at 37˚C or 30˚C (*Y. entero-colitica*) for 24h before each experiment. The *C. jejuni* strain was kept in freeze-stock at SVA. This was therefore plated on blood agar and incubated for at 41.5˚C for 48h in microaerophilic conditions. Before being used in the experiment bacteria were then transferred to a new blood agar plate and incubated at 41.5˚C for 24h.

Table 2. Bacterial strains and their origin.

Bacteria	**Strain**	**Origin**
Escherichia coli	ATCC 35218	Animal (Canine)
Staphylococcus aureus	CCUG 4151	Animal
Listeria monocytogenes	CCUG 15527	Animal
Salmonella Typhimurium*	CCUG 31969	Animal (Bovine liver)
Campylobacter jejuni	CCUG 11284	Animal (Bovine faeces)
Yersinia enterocolitica	CCUG 8239	Human (Faeces)

**Salmonella enterica* serotype Typhimurium

3.1.2 Determining bacterial concentrations

Pre-experimental trials were done to determine the concentration at an OD600 value of 0.1 in the spectrophotometer. Fresh bacterial culture material was added to buffered peptone water (BPW) and diluted until reaching the OD600 value between 0.100 and 0.160. A dilution-series with BPW as dilution medium was done by transferring 100µl of the bacterial solution to 900µl of BPW in serial dilutions. The dilution-series was made from 10^{1} to 10^{-8}. The dilutions 10^{-4} to 10^{-7} were streaked on blood agar plates and incubated at 37˚C for 24 hours and a viable count in CFU/ml was calculated for the original sample the next day. The blood agar plates for Campylobacter and Yersinia were instead incubated at 41.5˚C and 30˚C respectively for 48 hours. This knowledge of an approximate bacterial concentration at the OD600 value 0.1 was used in the experiments when making specific bacterial concentrations for inoculation of the cutting boards. For every bacterial strain the equation below was used to calculate how to produce a solution with 1 x 10^{7} CFU/ml, which was the starting solution for the dilution series used in the experiments (Table 3).

$$\frac{(1x10^{7}\ CFU/ml)}{Mean\ value\ of\ viable\ count}\ x\ 1000\mu l$$

This pre-experimental trial was done for each of the bacterial strains except for *C. jejuni* where no valid viable count could be evaluated due to the fluidity of the colonies. It was instead determined to use the OD600 value of 0.2 corresponding to 10^{9} CFU/ml (Krüger *et al.* 2014).

The dilutions for the experiments were done in autoclaved beef meat juice produced in the Substrate department at SVA. Fresh bacterial culture was added until the OD600 value between 0.100 and 0.160 was reached. The equation above was then used to make a solution of beef meat juice with 10^{7} CFU/ml. This 10^{7} CFU/ml solution was diluted from 10^{1} – 10^{-5} representing concentrations of 10^{7} – 10^{2} CFU/ml. The dilutions 10^{-2} – 10^{-5} were streaked on blood agar plates, incubated at 37˚C for 24 hours and a viable count was calculated for the original sample the next day. The blood agar plates for Campylobacter and Yersinia were instead incubated at 41.5˚C under micro-aerophilic conditions and 30˚C respectively for 48 hours.

Table 3. A list of the bacterial strains, the OD600 value used in the experimental designs and the mixture used to make a suspension with the correct bacterial concentration.

Bacteria	**OD600-value**	**Mixture**
Escherichia coli	0.101	79.9µl of bacterial suspension to 923.1µl of sterile beef juice
Staphylococcus aureus	0.106	71.5µl of bacterial suspension to 928.5µl of sterile beef juice
Listeria monocytogenes	0.156	43.5µl of bacterial suspension to 956.5µl of sterile beef juice
Salmonella Typhimurium*	0.121	66.7µl of bacterial suspension to 933.3µl of sterile beef juice
Campylobacter jejuni	0.2	10µl of bacterial suspension to 990µl of sterile beef juice
Yersinia enterocolitica	0.121	55.5µl of bacterial suspension to 944.5µl of sterile beef juice

3.1.3 Cutting board preparation

Three different commercial cutting boards (IKEA, Sweden) were used in the experiment. “APTITLIG”, 45x28cm, a wooden cutting board made of bamboo, “PROPPMÄTT”, 45x28cm, a wooden cutting board made of beech and “FINFÖRDELA”, 28x36xm, a plastic cutting board made of polypropylene.

The following procedures were performed in the typical experiment, any exception is indicated. Before inoculation, the cutting boards were rinsed under running, hot tap water for 10s (5s for each side), they were then scrubbed with a new dish sponge and commercial detergent liquid (YES original) for 40s (20s for each side). The same dish sponge was used for both sides of the cutting board. The cutting board was then rinsed under running, hot tap water again for 10s (5s for

each side). After the washing procedure the wooden cutting boards were put in a laminar flow safety cabinet to air dry for 20 ± 2 hours, UV-light were used on the cutting boards for the first hour of the drying period. The same washing protocol was used for each cutting board, the washing procedure for all cutting boards was done by the same person wearing nitrile gloves.

The cutting boards were marked with circles for control and inoculation. A control sample of the boards was then taken before inoculation by swabbing parts of the surface (not the marked areas) of each board with two swabs. The swabs were sterile cotton swabs moistened with autoclaved sterile water and the samples were streaked on blood agar plates directly, one for each board, and then incubated at 37˚C for 24h. The plates were then checked for growth.

3.1.4 Inoculation of cutting boards

The following procedures were performed in the typical experiment, any exception is indicated. The solutions for inoculations were prepared with premade beef meat juice from the Substrate department of SVA. In short, the beef meat juice was produced by mixing boneless beef inside/outside with water and heat it to 70˚C for 30 minutes. The meat juice was collected and then autoclaved at 121 ± 2˚C for 60 minutes, this procedure was repeated two times.

For each experimental design one of each of the three different kinds of cutting boards were used. The dilution series was done in sterile beef meat juice as described above. The marked areas of the cutting boards were each inoculated with 100µl representing 10, 100, 1,000 and 10,000, 100,000 and 1,000,000 CFU. A 100µl control with beef meat juice without bacteria was also added to each cutting board. The marked areas were covered with empty petri dishes to prevent contamination from the air (Figure 1.). The boards were kept and worked with in the safety cabinet until the inoculation was done and were then taken out and left on the lab bench for 24 ± 2 hours.

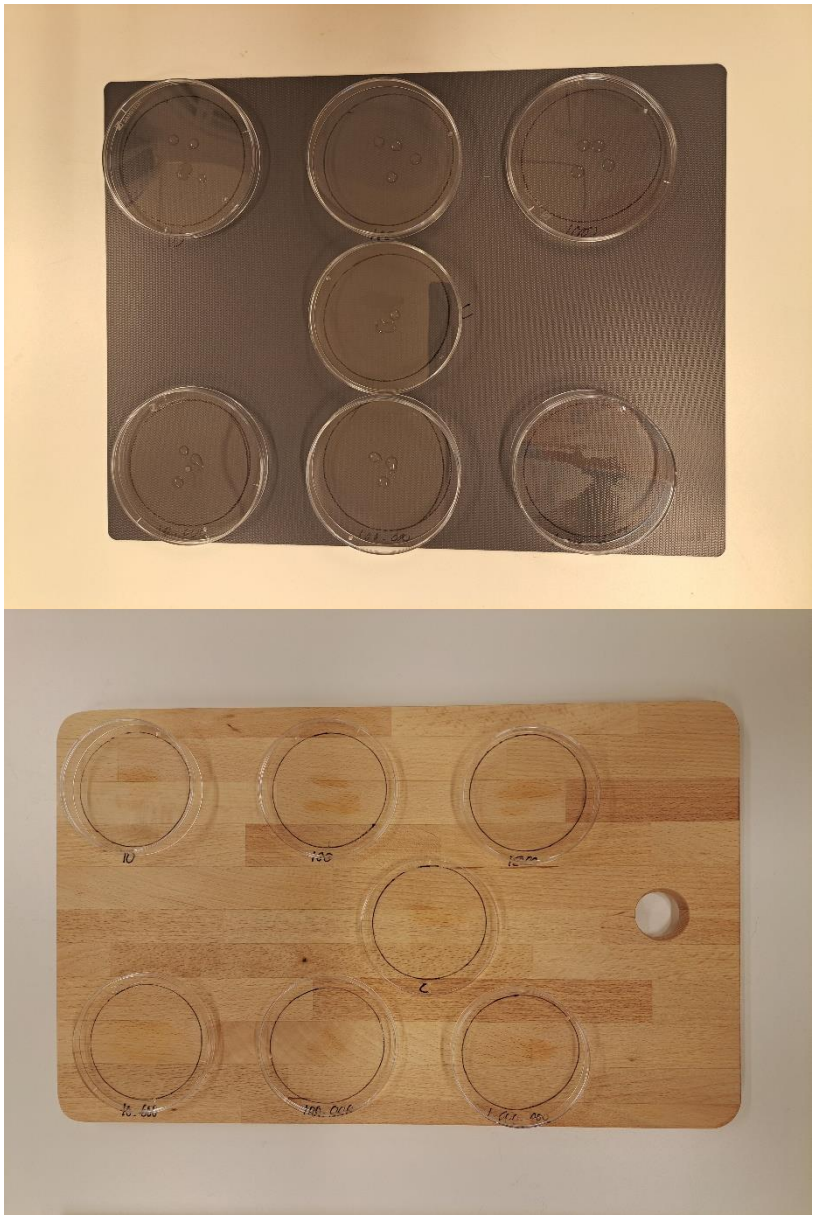


Figure 1. Inoculated plastic and beech cutting boards covered with empty petri dishes. Image created by the author.

3.1.5 Sample collection and analysis

The following procedures were performed in the typical experiment, any exception is indicated. After 24 ± 2 hours of incubation, sterile cotton swabs moistened with autoclaved sterile water and dragged in a zig-zag pattern over the marked areas. This was done two times for each circled area, with different swabs and in two different directions (Figure 2.). The samples from the swabs were directly spread out on blood agar plates or bromocresol purple (BCP) lactose agar depending on bacterial species used, one plate for each marked area. The plates were then incubated at 37˚C for 24 hours. For *C. jejuni* and *Y. enterocolitica* the plates were

instead incubated at 41.5˚C in micro-aerophilic conditions and 30˚C respectively for 48 hours. Growth or now growth was registered.

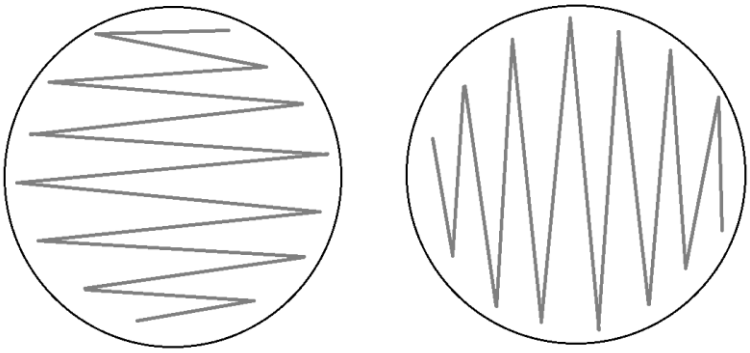


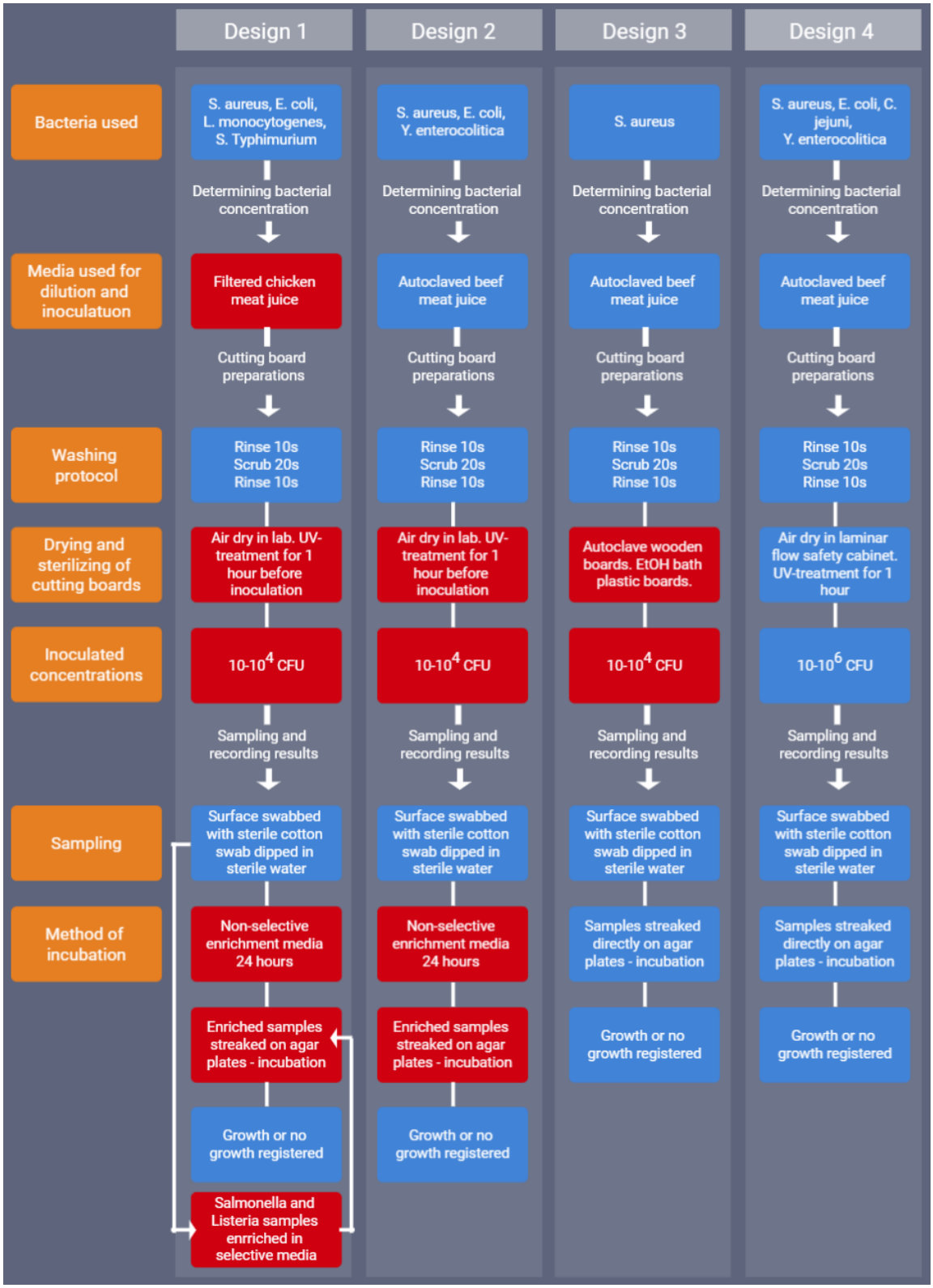
Figure 2. Image of the sampling technique used for the marked areas of the cutting board. Illustration created by the author.

3.2 Experimental designs

3.2.1 Summary of the experimental designs

The major differences between the designs of experiments are summarised in a flowchart below (Table 4) to simplify the procedure for the reader. More detailed information on each of the experimental designs can be found under "Investigation of background contamination" and "Experiments with functional laboratory protocol".

Table 4. Summary of the experimental designs. A flowchart presenting the major differences in the protocol between the different designs. Design 4 represents the finished protocol, areas marked red under the other designs show which steps of the designs differed from the finished protocol.



Flowchart created with https://www.visme.co/

3.2.2 Investigation of background contamination

Experimental design one

The first experimental design was done with *S. aureus*, *E. coli*, *S.* Typhimurium and *L. monocytogenes*. The preparation of bacterial cultures and determination of bacterial concentrations was done as described in "Preparation of bacterial cultures" and "Determining bacterial concentrations", except chicken meat juice was used for the dilution series. Swedish frozen chicken breast filets (Kronfågel) were purchased from a grocery store. The chicken breasts were thawed in a refrigerator for 48h, the meat juice was then collected with a sterile syringe, dispensed in tubes and centrifuged at 4302 RCF for 10 minutes. The supernatant was transferred to 2ml Eppendorf tubes and centrifuged again at 12000 RCF for another 10 minutes. The supernatant from the second centrifugation was then filtered through a syringe filter with a pore size of 0.2µm (Figure 3) and stored in 2ml tubes in a freezer. As a contamination control, 100µl of filtered chicken meat juice was spread on two blood-agar plates. One blood agar plate was incubated at 37°C for 24h and the other in 41.5°C for 48h under micro-aerophilic conditions, they were then checked for growth. The filtered chicken meat juice was also used for inoculating the cutting boards.

For the cutting board preparation, the same washing protocol as described in the "Cutting board preparation". The cutting boards were then left to air dry in the lab for at least one hour and then stored together in a plastic bag until the day they were to be inoculated. At the day of inoculation, the cutting boards were marked with five circles using a marker pen representing control, 10, 100, 1000 and 10.000 CFU respectively (Figure 4). The cutting boards were treated under UV-light for one hour and then inoculated with bacteria. The marked areas were not covered, and the cutting boards were left on the lab-bench for 24 hours before sampling an analysis were done.

The sampling method for *E. coli* and *S. aureus* was performed as described in "Sample collection and analysis" three cotton swabs were used instead of two. The swabs were then put in tubes with 10ml, non-selective nutrient broth as enrichment media and enriched in an incubator at 37°C for 20-24 hours. After the enrichment each sample was spread on two blood agar plates, one by using a 10µl loop and one by spreading 100µl of the sample on the plate. The plates were then incubated at 37°C for 24 hours and growth or no growth was registered. The sampling method for *S.* Typhimurium and *L. monocytogenes* was initially the same as the ones mentioned above, with three cotton swabs used. The cotton swabs were then sent to the department of bacteriology on SVA to be enriched and cultivated according to ISO-standard 6579-12017 for *S.* Typhimurium and an accredited modified international IDF-standard 143A:1995 for *L. monocytogenes*. Growth or no growth was registered.

To investigate possible sources of contamination, samples were taken from the sterile water used for the cotton swabs, from the chicken meat juice, from the chicken meat juice combined with nutrient broth and from the nutrient broth. For the sterile water and the chicken meat juice the samples were plated directly on blood agar plates and incubated at 37°C overnight. For the chicken meat juice combined with nutrient broth and the nutrient broth both were enriched in the incubator at 37°C for 24 hours and then plated on blood agar plates and incubated at 37°C overnight.

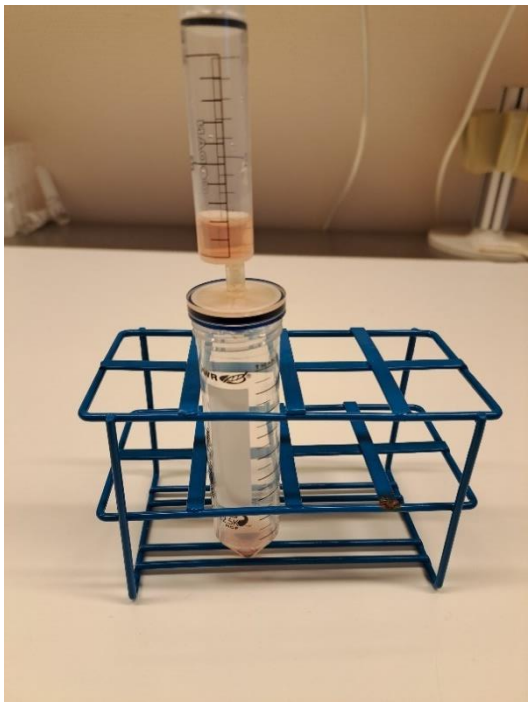


Figure 3. Image of chicken meat juice being filtered through a syringe filter. Image created by the author.

Experimental design two

The second experimental design was done with *E. coli*, *S. aureus* and *Y. enterocolitica*. The preparation of bacterial cultures and determination of bacterial concentrations was done as described in "Preparation of bacterial cultures" and "Determining bacterial concentrations". The cutting board preparation was done in the same way as in the first experimental design. After inoculation the marked areas of the cutting board were covered with empty petri dishes to minimize possible environmental contamination. The cutting boards were left on the lab-bench for 24 hours before sampling. The sampling was performed as described in "Sample collection and analysis". The swabs were then put in tubes with 10ml, non-selective nutrient broth (*E. coli* and *S. aureus*) or 10ml, non-selective brain-heart-infusion broth (*Y. enterocolitica*) as enrichment media and enriched in an incubator at 37°C (*E. coli* and *S. aureus*) or 30°C (*Y. enterocolitica*) for 20-24 hours. After the enrichment step, the samples were plated on blood agar (*S. aureus* and *Y. entero-colitica*) or BCP lactose agar (*E. coli*) plates and incubated at 37°C for 24 hours (*E.*

coli and *S. aureus*) or 30˚C for 48 hours (*Y. enterocolitica*). Growth or no growth was registered.

To investigate possible sources of contamination, sampling of the lab environment was done by taking the lid of two blood agar plates and letting them stand in the lab for 24 hours, on the lab bench and sink respectively. Another environmental sample was also taken the same way by putting a blood agar plate in the safety cabinet where the cutting boards were UV-treated. The plates were then incubated at 37˚C for 24 hours. Sampling of the cutting boards after the washing procedure but before the UV-light treatment was done by dipping a sterile cotton swab in sterile water and moving it in a zig-zag pattern over the board, this was done two times and the samples was then directly spread on blood agar plates and incubated at 37˚C overnight. The surfaces of the cutting boards were then sprayed with 75% EtOH solution and sampled again the next morning, the samples were incubated at 37˚C overnight.

Samples were then also taken of the water used to wash the boards and of the boards before the washing procedure and incubated at 37˚C overnight.

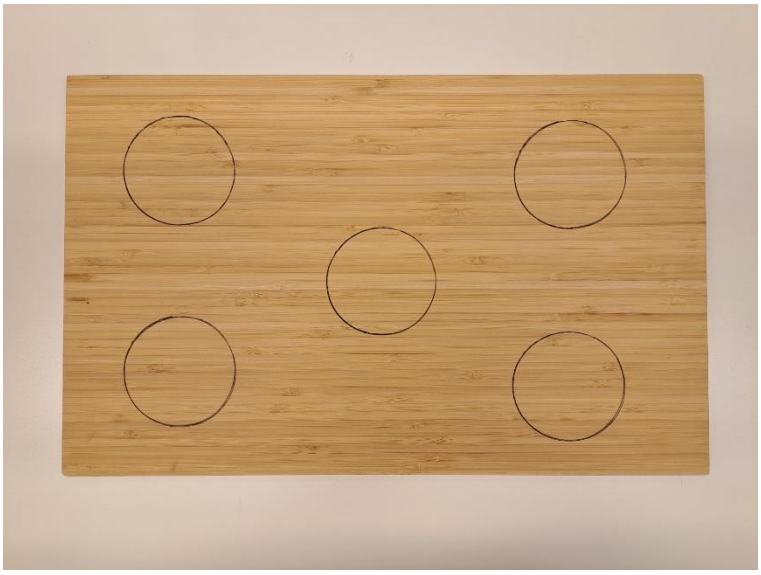


Figure 4. Image of bamboo cutting board with marked areas for inoculation. Image created by the author.

Experimental design three

The third experimental design was done with only *S. aureus*. The preparation of bacterial culture and determination of bacterial concentration was done as described in "Preparation of bacterial cultures" and "Determining bacterial concentrations". The washing procedure of the cutting boards was performed as described in "Cutting board preparations". The wooden cutting boards was then covered with aluminium foil and autoclaved at 121˚C for 15 minutes while the plastic cutting board was put in a 70% EtOH both for 5 minutes and left to air dry in a laminar flow safety cabinet. The beech wood cracked and bent from the autoclaving procedure, but the bamboo wood tolerated it better and did not crack or change shape.

The markings on the cutting boards were drawn on after the autoclaving and EtOH bath, representing control, 10, 100, 1,000 and 10,000 CFU.

To investigate possible sources of contamination, samples were taken from each of the three cutting boards before inoculation. The samples were taken with two cotton swabs dipped in sterile water and were then streaked directly on to blood agar plates and inoculated at 37°C overnight. The cutting boards were then inoculated with *S. aureus*, the marked areas were covered with empty petri dishes and the cutting boards were left on the lab bench for 24 hours. The bacterial samples were then taken as described in "Sample collection and analysis", streaked directly on to blood agar plates and incubated at 37°C for 24 hours. No enrichment was done on the samples.

3.2.3 Experiments with a functional laboratory protocol

Experimental design four

The fourth experimental design was done with *S. aureus*, *E. coli*, *Y. enterocolitica* and *C. jejuni*. The preparation of bacterial culture and determination of bacterial concentration was done as described in "Preparation of bacterial cultures" and "Determining bacterial concentrations". The washing procedure of the cutting boards was performed as described in "Cutting board preparations" and the boards were left to air dry in a laminar flow safety cabinet. The boards were then marked with seven circles representing control, 10, 100, 1,000, 10,000, 100,000 and 1,000,000 CFU. The boards were swabbed for contamination control, the swabs were streaked on a blood agar or BCP lactose agar plate for each type of cutting board and incubated overnight at 37°C for *E. coli* and *S. aureus*, 30°C for *Y. entero-colitica* and 41.5°C in micro-aerophilic conditions for *C. jejuni*. Blood agar was use for *S. aureus* and C. *jejuni* and BCP lactose agar was used for *E. coli* and *Y. enterocolitica*. The marking and inoculation of the boards were done in a laminar flow safety cabinet. The marked areas were then covered with empty petri dishes and the boards were left on a lab bench for 24-48 hours. The sampling was done as described in "Sample collection and analysis" and growth or no growth was registered after 24 hours. No enrichment was done one the samples. The laboratory protocol (Appendix 1) was adapted to follow the procedure from experimental design four.

4. Results

4.1 Background contamination

In the first experimental design, background contamination was found in all the samples for *S. aureus* (Table 6) and *E. coli* (Table 5). The samples from *S.* Typhimurium (Table 7) and *L. monocytogenes* (Table 8) were enriched and cultivated in selective broth and on selective plates, therefore it is not confirmed but highly likely that the background contamination was present also in these samples. Samples were taken from the sterile water used for the cotton swabs, from the chicken meat juice, the nutrient broth and from the chicken meat juice combined with nutrient broth. Only the samples of chicken meat juice and chicken meat juice combined with nutrient broth showed growth. Before the experiments started, a control sample of the chicken meat juice had been cultivated with no growth. By these results it was concluded that the chicken meat juice was a source of background contamination, and the experiments was continued with autoclaved sterile beef meat juice instead.

In the second experimental design the contamination was still present but more sporadic and not in every sample taken for *E. coli* (Table 9), *S. aureus* (Table 10) and *Y. enterocolitica* (Table 11). This concluded that the chicken meat juice most likely was one, but not the only source of contamination in the experiments. Two environmental samples from the lab and one from the safety bench where the cutting boards were UV-treated were taken. In the samples from the lab there were growth of bacteria. The sample taken from the water, used to wash the cutting boards, showed no signs of growth. The boards were also swabbed and sampled after the washing procedure when they had dried and after that the surface was sprayed with 75% EtOH and the cutting boards were sampled again. The boards showed a lot of growth of background flora after the washing procedure. After the EtOH treatment the contamination was less but still present. It was concluded that there could be contamination from the air on the cutting board while they air dried in the lab. A new approach was therefore tested for sterilising the cutting boards. For third experimental design the wooden cutting boards were autoclaved and the plastic cutting boards were put in a 70% EtOH bath. No contamination was found on the boards after this treatment but one of the wooden cutting boards cracked and bent from the autoclaving and this protocol was there fore discontinued.

The procedure for drying the cutting boards, that was adapted into the final laboratory protocol, was to let the cutting boards air dry in a laminar flow safety cabinet. Control samples taken from each of these boards before inoculation showed no contamination.

Table 5. Results from experimental design one, cutting boards were inoculated with E. coli. *The OD600 value was 0.107 and the bacterial concentration in the original solution was calculated to be 9.7 x 10^6.*

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	M	M	M
10 CFU	+ M	M	+ M
10^2 CFU	+ M	M	+ M
10^3 CFU	+ M	+ M	M
10^4 CFU	+ M	M	+ M

M = mixed background flora
+ = growth of the inoculated bacteria

Table 6. Results from experimental design one, cutting boards were inoculated with S. aureus. *The OD600 value was 0.107 and the bacterial concentration in the original solution was calculated to be 1.2 x 10^7.*

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	M	M	M
10 CFU	M	M	M
10^2 CFU	+ M	+ M	M
10^3 CFU	+ M	M	M
10^4 CFU	+ M	M	M

M = mixed background flora
+ = growth of the inoculated bacteria

Table 7. Results from experimental design one, cutting boards were inoculated with S. *Typhimurium. The OD600 value was 0.124 and the bacterial concentration in the original solution was calculated to be 1.1 x 10^7.*

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	NG	NG	NG
10 CFU	NG	NG	NG
10^2 CFU	NG	NG	NG
10^3 CFU	+	NG	NG
10^4 CFU	+	NG	+

NG = no growth
+ = growth of the inoculated bacteria

Table 8. Results from experimental design one, cutting boards were inoculated with L. monocytogenes. *The OD600 value was 0.159 and the bacterial concentration in the original solution was calculated to be 4.4 x 10^6.*

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	NG	NG	NG
10 CFU	NG	NG	NG
10^2 CFU	+	NG	NG
10^3 CFU	+	NG	NG
10^4 CFU	NG	NG	NG

NG = no growth
+ = growth of the inoculated bacteria

Table 9. Results from experimental design two, cutting boards were inoculated with E. coli. *The OD600 value was 0.099 and the bacterial concentration in the original solution was calculated to be 9.2 x 10^6.*

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	+ M	NG	NG
10 CFU	M	M	NG
10^2 CFU	M	NG	M
10^3 CFU	+ M	NG	M
10^4 CFU	+ M	NG	M

NG = no growth
M = mixed background flora
+ = growth of the inoculated bacteria

Table 10. Results from experimental design two, cutting boards were inoculated with S. aureus. *The OD600 value was 0.107 and the bacterial concentration in the original solution was calculated to be 5.6 x 10^6.*

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	M	M	M
10 CFU	NG	M	M
10^2 CFU	NG	NG	M
10^3 CFU	+ M	M	M
10^4 CFU	+ M	+ M	M

NG = no growth
M = mixed background flora
+ = growth of the inoculated bacteria

Table 11. Results from experimental design two, cutting boards were inoculated with Y. enterocolitica. *The OD600 value was 0.126 and the bacterial concentration in the original solution was calculated to be 1.2 x* 10^7.

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	M	NG	NG
10 CFU	M	NG	M
10^2 CFU	M	NG	NG
10^3 CFU	+ M	NG	NG
10^4 CFU	+ M	NG	NG

NG = no growth
M = mixed background flora
+ = growth of the inoculated bacteria

4.2 Without background contamination

During experimental design three to four there were no instances of background contamination. The results are shown in table 12 to 16. Experimental design three was limited to only include *S. aureus* due to the need of confirming the samples being clear of background contamination. Compared to the early experiment with *S. aureus* the later ones showed 10 (Table 12) and 100 (Table 13) times decreased cut-off value. One of the later experiments also showed that *S. aureus* was detectable at the surface of the bamboo cutting board when inoculated at high concentrations (Table 13). The treatment of the cutting boards differed in experimental design three as explained in method and materials and was discontinued. Experimental design four followed the same laboratory protocol and was done for *S. aureus*, *E. coli*, *Y. enterocolitica* and *C. jejuni*. The experimental designs three and four differed mostly from the earlier ones in that no enrichment was used for the samples, they were instead streaked directly on agar plates. No growth for any of the bacterial strains could be seen on beech when using the direct agar plate method (Table 12-16). On bamboo the only positive samples with the agar plate method were for *S. aureus* at inoculated concentrations of 10^5 and 10^6. For the plastic cutting boards, all the strains were positive at 10^5 and 10^6, E. coli was detectable at the lowest inoculated concentration of 10^2.

Table 12. Results from experimental design three, cutting boards were inoculated with S. aureus. *The OD600 value was 0.107 and the bacterial concentration in the original solution was calculated to be 6.9 x 10^6. No contaminating flora was observed.*

Inoculation	Plastic	Beech	Bamboo
Control	NG	NG	NG
10 CFU	NG	NG	NG
10^2 CFU	NG	NG	NG
10^3 CFU	+	NG	NG
10^4 CFU	+	NG	NG

NG = no growth
+ = growth of the inoculated bacteria

Table 13. Results from experimental design four, cutting boards were inoculated with S. aureus. *The OD600 value was 0.103 and the bacterial concentration in the original solution was calculated to be 7.2 x 10^7. No contaminating flora was observed.*

Inoculation	Plastic	Beech	Bamboo
Control	NG	NG	NG
10 CFU	NG	NG	NG
10^2 CFU	NG	NG	NG
10^3 CFU	NG	NG	NG
10^4 CFU	+	NG	NG
10^5 CFU	+	NG	+
10^6 CFU	+	NG	+

NG = no growth
+ = growth of the inoculated bacteria

Table 14. Results from round four, cutting boards were inoculated with E. coli. *The OD600 value was 0.100 and the bacterial concentration in the original solution was calculated to be 8.6 x 10^7. No contaminating flora was observed.*

Inoculation	Plastic	Beech	Bamboo
Control	NG	NG	NG
10 CFU	NG	NG	NG
10^2 CFU	+	NG	NG
10^3 CFU	+	NG	NG
10^4 CFU	+	NG	NG
10^5 CFU	+	NG	NG
10^6 CFU	+	NG	NG

NG = no growth
+ = growth of the inoculated bacteria

Table 15. Results from experimental design four, cutting boards were inoculated with Y. enterocolitica. *The OD600 value was 0.122 and the bacterial concentration in the original solution was calculated to be 2.6 x 10^7. No contaminating flora was observed.*

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	NG	NG	NG
10 CFU	NG	NG	NG
10^2 CFU	+	NG	NG
10^3 CFU	+	NG	NG
10^4 CFU	+	NG	NG
10^5 CFU	+	NG	NG
10^6 CFU	+	NG	NG

NG = no growth
+ = growth of the inoculated bacteria

Table 16. Results from experimental design four, cutting boards were inoculated with C. jejuni. *The OD600 value was 0.196 and the bacterial concentration in the original solution was calculated to be 1.4 x 10^7. No contaminating flora was observed.*

Inoculation	**Plastic**	**Beech**	**Bamboo**
Control	NG	NG	NG
10 CFU	NG	NG	NG
10^2 CFU	NG	NG	NG
10^3 CFU	NG	NG	NG
10^4 CFU	NG	NG	NG
10^5 CFU	+	NG	NG
10^6 CFU	+	NG	NG

NG = no growth
+ = growth of the inoculated bacteria

5. Discussion

Problem with background contamination was present in the early experimental designs this forced us to change our protocol and try different experimental designs. Identified probable sources were, both the filtered chicken meat juice used in the early experiments and environmental contamination of the boards. The contamination of the boards was traced back to the air drying of the washed boards in ordinary laboratory room environment. This was concluded since there was next to no bacteria growing on the new, none washed boards but a significant amount of bacteria growing on the boards after the washing and drying procedure.

At the start of the study we hoped to be able to do three experiments for each bacterial species with a functioning protocol. Due to the unexpected difficulties with the background contamination, the time designated for this study were not enough to do all the replications we wanted. At the start of the study, the plan was for the samples of *S*. Typhimurium, *C. jejuni* and *L. monocytogenes* to be analysed at routine lab at SVA and the samples of *E. coli*, *Y. enterocolitica* and *S. aureus* to be analysed by the author. Due to practicalities the first experimental design was set up so to include two bacterial species that were to be analysed at routine lab and two by the author. The routine lab analysis was discontinued after this due to the bacterial contamination event which led to us wanting to exclude parts of the design which we did not have full control over ourselves. When the functional laboratory protocol was presented, there was unfortunately no more time for samples to be sent to routine lab. In the later experimental designs, the choice of bacterial species to include was based on which bacteria was easiest to work with and also by the fact that we wanted to include all our chosen bacterial species at least once to give the author experience in working with these different bacterial species in laboratory environment.

After we started drying the boards in a laminar flow safety cabinet, the problem with background contamination disappeared. Even though the kitchen environment in a domestic setting is most likely not sterile we wanted to have a sterile setting so to properly be able to compare the difference in bacterial survival on the different cutting board materials. We also wanted to keep the treatment to the boards as minimal as possible as to not interfere with the properties of the materials used. Also, boards in domestic settings most likely is not treated with EtOH or autoclaved and we wanted to keep it as close to these conditions as possible. The difficulty

with sterilising the cutting boards was not expected and shows that air drying the boards in room environment could be a risk factor for background contamination in earlier and future studies of this kind.

The filtered chicken meat juice that was also a source of contamination was primarily used for the proteins and other nutrient factors that would support the survival of the inoculated bacteria and at the same time be close to the conditions that could be present in a domestic setting. Unfortunately, we did not manage to get it as sterile as we needed. It might have been due to wrong handling of the filtration in the lab that made the chicken juice not completely sterile, but we did not manage to conclude the source of the contamination.

In the early experimental designs, the samples were incubated in enrichment media before being plated on agar plates. In the later experimental designs, the samples were streaked directly on agar plates with no enrichment. The enrichment method could confirm growth of an inoculated *E. coli* strain down to 10 CFU even though background contamination was present. With the direct agar method, the lowest concentration where bacteria could be detected was 100 CFU for *E. coli* and *Y. enterocolitica*.

In all the experiments where no contamination was present, and the samples were streaked directly on agar plates it was consistently more growth on the plastic cutting boards than on the wooden cutting boards. This result was independent of what bacterial strain was used even though *E. coli* and *Y. enterocolitica* was found on the areas inoculated with lower concentration of bacteria than was *C. jejuni* or *S. aureus*. A study by Schönwälder *et al.* (2002) where they tested the survival of *E. coli*, showed that the reduction of bacteria on the surface of plastic cutting boards started first after 12-24 hours while the reduction of bacteria on the wooden cutting boards started after about 1-2 hours when the inoculum had dried up. After 24 hours viable bacteria could still be detected on the surface of plastic boards and on the surface of beech and poplar wood but not on pine wood. Ak *et al.* (1994) showed that bacteria could still be detected on the surface of wooden cutting boards after 12 hours but that there had been a reduction of bacteria by 98-99.9%. This was independent of the type of wood used. In the same study they also had a four time increase in bacteria on the plastic cutting boards after 12 hours. This also correlates to the results from our study where bacteria were detectable more often on the surface of plastic boards than wooden boards. In our study there was also a difference between beech and bamboo where bacteria were detectable more often on the surface of the bamboo boards. The exception was the early experiments with *S. aureus* using the enrichment method where bacteria was detectable on beech but not on bamboo. These experiments included background contamination and the factor of competition between bacterial species therefore it is hard to make any conclusions for these findings.

The study by Schönwälder *et al.* (2002) also showed that the higher the initial concentration of bacteria was the longer they could be detected, and that it decreased faster on wood than on plastic. If only low concentrations of bacteria were applied (10^3-10^4 CFU/cm2) the bacteria behaved similarly independent of material. In our study the lowest detectable concentration on plastic after 24 hours by the direct agar plating method was 100 CFU. No samples from the wooden boards for any of the bacterial strains tested had any bacterial growth at such low concentration with the direct agar plate method, the lowest concentration registered was 10^5 for *S. aureus* on bamboo. This could have several explanations; with the small inoculum volume used in our experiment the surface most likely dry out fast and gets more inhospitable for the bacteria. The specificity of the cultivation method could also be a factor, especially since it was possible to isolate bacteria from the lower inoculum concentrations on wood when we used the enrichment method. The difference in results depending on which method was used was most apparent in *E. coli*.

In the same study by Schönwälder *et al.* (2002) they also showed that bacteria translocated from the surface to the inner structures of wood and that some types of wood i.e., pine wood seemed to inhibit antibacterial properties because the reduction of bacteria on both the surface and inner structures was marked compared to beech and other types of wood used in the study. This possible translocation of bacteria could be another factor to why it was harder to isolate bacteria from the surface of the wooden cutting boards in our own experiments. However, in the kitchen situation bacteria translocated to the inner structure of wooden cutting boards might not pose a risk, unless they migrate to the surface.

The results from this study show that bacteria could be detected more often on plastic than wooden cutting board surfaces. It therefore supports that there are a difference in the survival of bacteria on the surface of wooden and plastic cutting boards. It also indicates a difference between beech and bamboo cutting boards where bacteria is detected more often on bamboo than on beech, though this conclusion would need to be strengthened through further studies. The working laboratory protocol is presented both in Material and methods and summarized in Appendix 1. The timeframe of this study and the initial problems with background contamination did not support enough replications of the experiments. The validity of the data could therefore be questioned and should preferably be validated through further studies. This study was instead focused more on presenting a functional laboratory protocol that could be used in future studies for this purpose. Continuing studies could very well do the same type of experiment to confirm the results or focus more on the difference in survival between different bacterial strains. This information could be interesting to have since the bacteria differ in how well they survive in varying storing temperatures and their infectious dose. The laboratory

protocol could also be modified to compare methods using enrichment to methods using only direct plating.

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Popular Science Summary

Illness caused by food contaminated with disease causing bacteria, so called foodborne illness is a common problem globally. In 2010 an estimated number of 600 million people were affected by foodborne illness. Depending on different factors the type of disease caused can vary from mild; headache and vomiting for a few hours, to so severe as to lead to death. These factors include type of contaminating bacteria, how good the immune system of the person ingesting the bacteria is and the possibility of good health care.

Disease-causing bacteria can contaminate food in many ways, one of the most common being during the food preparation in our own private households. It is not always easy to know how your food get contaminated, and it is often not apparent to the naked eye that the food is contaminated with disease-causing bacteria. Contamination can occur by bad hygiene routines, by processing contaminated raw ingredients and by improper storing of food and food products. Kitchen utensils is one common way for bacteria to cross-contaminate different food products. If you for example cut chicken on a cutting board and right after, without cleaning the knife or the cutting board you start cutting lettuce for a salad there is a risk that you transfer disease-causing bacteria from the chicken to the salad. If you forget to wash the cutting board after cutting the chicken and then use the cutting board to cut some other food the next day, would there still be a risk that the disease-causing bacteria survived on the cutting board during these hours and end up in your food? This study has shown that it is possible for bacteria to survive on the surface of cutting boards for 24 hours depending on how high the concentration of bacteria was from the start and what type of cutting board was used. Bacteria could be isolated from the surface of plastic cutting boards more often than from wooden cutting boards. This study also presented a laboratory protocol that could be used in further research experiments to study the survival of bacteria on the surface of cutting boards of different materials.

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Appendix 1

Laboratory protocol

This laboratory protocol is a simplification of the method and materials description. It is meant to be used during laboratory work to make it easy to follow the steps.

Day 1

Needed materials

- 3 cutting boards (beech, bamboo, plastic)
- 1 blood agar or BCP lactose agar plate
- Reference bacterial strain
- Laminar flow safety cabinet
- Sink with hot tap water
- Nitrile gloves
- Dish sponge and detergent
- UV-light
- Incubator (30, 37 or 40.5C)
- Anoxomat (For C. jejuni)

Cutting board preparations

- Take three new boards for each bacterial strain used in this round of experiment: beech, plastic, bamboo
- Wash one board at a time, use a timer and follow the following protocol, use gloves.
  - Rinse under hot tap water for 10 seconds (5 seconds for each side)
  - Scrub for 40 seconds (20 seconds for each side) with a dish sponge and a string of detergent
  - Rinse under hot tap water for 10 seconds (5 seconds for each side)
  - Put the board in a clean dish rack
- Place in a laminar flow safety cabinet directly after washing and allow to dry. The boards are to be UV treated for the first hour of the drying time.

Bacterial culture preparations

- Incubate each bacterial strain on either blood agar or BCP lactose agar (*E. coli*, *Y. enterocolitica*).
- Incubate the plates according to the instructions in Table 1a.

Table 1a. Bacterial strains and their growth conditions

Bacteria	**Strain**	**Growth conditions**
Escherichia coli	ATCC 35218	37C, 24h
Staphylococcus aureus	CCUG 4151	37C, 24h
Listeria monocytogenes	CCUG 15527	37C, 24h
Salmonella Typhimurium*	CCUG 31969	37C, 24h
Campylobacter jejuni	CCUG 11284	41.5C, micro-aerophilic conditions, 48h
Yersinia enterocolitica	CCUG 8239	30C, 48h

**Salmonella enterica* serotype Typhimurium

Day 2

Needed materials

- Agar plate with respective bacterial strain
- 1µl white loop
- Spreader
- 25ml Dilution media (sterile beef juice)
- 1 sterile glass cylinder 25ml
- 1 sterile test tube 10ml
- 6 sterile Eppendorf tubes 1.5ml
- Marker pen
- 8 blood agar plates
- Laminar flow safety cabinet
- Spectrophotometer
- Glass cuvettes
- 100-1000µl pipette and pipette tips
- 10-100µl pipette and pipette tips
- 11 Empty petri dishes
- Sterile (autoclaved) water 2ml

Determining bacterial concentration

NOTE! If you are working with *S.* Typhimurium, *L. monocytogenes* or *Y. enterocolitica*, the following steps should be carried out in a biosafety cabinet

- Transfer 25ml of sterile beef meat juice to a sterile glass cylinder
- Transfer 100µl of the sterile beef meat juice to a blood agar plate marked with "control", spread it using a spreader and incubate at 37C for 24 hours
- Put 7 sterile Eppendorf 1.5ml tubes in a holding rack, transfer 900µl of sterile beef juice to 6 of the tubes. Mark one of the tubes with C (for control) and the rest with -1 to -5.
- Add 2-4ml of sterile beef meat juice to a sterile test tube (mark original) and take a white loop of colony material from the bacterial strain you plated on day one. Resuspend completely, vortex if necessary.
- Add 1ml of suspension to a glass cuvette and measure the OD600 value in a spectrophotometer. The value should be similar (± 0.02) as the reference value indicated in table 2a.
  - If the OD600 value is lower – add more bacterial culture, vortex for a few seconds and measure again.
  - If the OD600 value is higher – add more sterile beef meat juice, vortex for a few seconds and measure again.
  - If the OD600 value is similar – continue to the next step
- Mark the remaining empty Eppendorf tube with 0
- Transfer the volume indicated in Table 2a from the original culture to the Eppendorf tube marked 0. Add the amount of sterile beef juice indicated in table 2a and vortex for a few seconds
- Add 900µl of sterile beef juice to each tube marked -1 to -5
- Do 10-fold serial dilution starting with tube 0. REMEMBER to vortex at each step
  - Transfer 100µl from tube 0 to tube -1
  - Transfer 100µl from tube -1 to tube -2
  - Transfer 100µl from tube -2 to tube -3
  - Transfer 100µl from tube -3 to tube -4
  - Transfer 100µl from tube -4 to tube -5

Table 2a. OD600 value in relation to growth for each bacteria

Bacteria	**OD600-value**	**Mixture**
Escherichia coli	0.101	79.9µl of bacterial suspension to 923.1µl of sterile beef juice
Staphylococcus aureus	0.106	71.5µl of bacterial suspension to 928.5µl of sterile beef juice
Listeria monocytogenes	0.156	43.5µl of bacterial suspension to 956.5µl of sterile beef juice
Salmonella Typhimurium*	0.121	66.7µl of bacterial suspension to 933.3µl of sterile beef juice
Campylobacter jejuni	0.2	10µl of bacterial suspension to 990µl of sterile beef juice
Yersinia enterocolitica	0.121	55.5µl of bacterial suspension to 944.5µl of sterile beef juice

Viable count

- Mark 4 blood agar plates with -2 to -5
- Transfer 100µl from the -2, -3, -4 and -5 dilutions and spread them out on one blood agar plat for each dilution using a spreader.
- Incubate the plates according to table 1a.

Inoculation of cutting boards

NOTE! These steps should be done in a laminar flow safety cabinet

- Use a marker pen and for example an empty petri dish, draw 7 circles on your cutting boards and mark them with C, 10, 100, 1000, 10.000, 100.000 and 1.000.000 respectively.
- Take two sterile cotton swabs, moisten them with sterile water and swab the areas of the cutting boards that are not marked. Streak the contamination-control samples directly onto a blood agar plate. Use two swabs and one blood

agar plate for each type of cutting board.
Incubate at 37C for 24 hours.

- Transfer 100µl from the tubes to the center of the marked areas. After each transfer, cover the area with an empty petri dish to minimize contamination.
  - Transfer 100µl from tube C to marked area C
  - Transfer 100µl from tube -5 to marked area 10
  - Transfer 100µl from tube -4 to marked area 100
  - Transfer 100µl from tube -3 to marked area 1000
  - Transfer 100µl from tube -2 to marked area 10.000
  - Transfer 100µl from tube -1 to marked area 100.000
  - Transfer 100µl from tube 0 to marked area 1.000.000
- Take the inoculated board with the petri dishes out of the laminar flow safety cabinet and put it on the bench. Let them stand at room temperature for 24 hours before sampling.

Day 3

Needed materials

- 21 blood agar plates or 21 BCP lactose agar plates (*E. coli* or *Y. enterocolitica*)
- 1 prepared cutting board of each material (bamboo, beech and plastic)
- Sterile (autoclaved) water 4ml
- Sterile cotton swabs

Sampling the cutting boards

- Take 7 blood agar plates or BCP lactose agar plate (*E. coli* or *Y. enterocolitica*) for each board and mark them with beech, bamboo or plastic and the corresponding number of the marked area.
- Fill a sterile test tube with sterile (autoclaved) water
- Use sterile cotton swabs, two for each marked area. Dip the cotton swab in the sterile water and drag it in a zig-zag pattern over the marked area (Figure 1a.).
- After you sampled the area with one cotton swab, take the swab and streak the sample out on half of the blood agar plate or BCP lactose agar plate (E. coli or Y. enterocolitica). Take the sample with the next swab on the same area and streak it out on the other half of the plate.
  Repeat for all the marked areas.
- Incubate as listed in Table 1a.

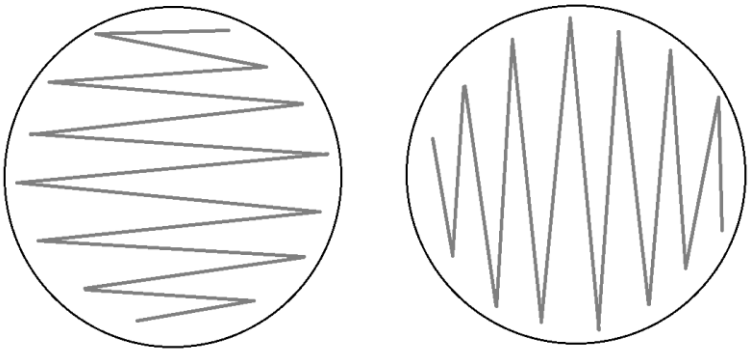

Figure 1a. *How to sample the marked areas*

Read the viable count plates

- Count the colonies on each plate for the bacterial strains incubated for 24h.
  Only use plates with a maximum of 300 colonies.
  Too numerous to count (TNTC) or no growth (NG) are registered for plates with over 300 colonies or plates with no growth at all.
- Calculate the concentration in the original sample for each dilution using the equation below then calculate the mean value.

$$CFU/ml = \frac{(number\ of\ colonies\ on\ plate)\ x\ (dilution\ facctor)}{(valume\ plated\ in\ ml)}$$

Day 4 and 5

Reading the plates

- **Reading plates**
  - After 24 hours read the plates from the sampling, growth or now growth is registered
  - After 48 hours read the plates from the sampling (*C. jejuni* and *Y. enterocolitica*), growth or no growth is registered

Read the viable count plates

- **Viable count**
  - Count the colonies on each plate for the bacterial strains incubated for 48h and calculate viable count using the same method described for bacterial strains incubated for 24h (above).

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