Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the equivalence between the disinfection of tools in slaughterhouses and cutting rooms with hot water supplied at a temperature not less than 82 ºC, and various alternative systems of disinfection

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Abstract

The different tools and utensils used in slaughterhouses and cutting rooms may lead to cross-contamination if unsuitable cleaning and disinfecting procedures are used. In this regard, Regulation (EC) No. 853/2004 laying down specific hygiene rules for food of animal origin establishes that slaughterhouses and cutting rooms for ungulates as well as poultry and lagomorphs must have facilities for disinfecting tools with hot water supplied at not less than 82 ºC, or an alternative system having an equivalent effect.

The Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) has

assessed several studies carried out in order to establish whether disinfection with four alternative systems may be considered equivalent to that conducted with water supplied at a temperature not less than 82 ºC.

After reviewing the studies, the AESAN Scientific Committee concludes that a notable effort has been made to demonstrate the equivalence of these systems. It is observed in these studies that the use of these compounds leads to reductions in the microorganisms studied which, under the testing conditions, appear to be similar to those obtained with the official method. However, these studies have methodological limitations (number of repetitions, sampling plan, the microorganisms studied and method of analysis) which prevent establishing this equivalence.

Accordingly, the Committee makes a series of recommendations for conducting these studies: using swabs instead of contact slides; including the analysis of foodborne pathogenic microorganisms of interest in slaughterhouses; including detailed information on the disinfecting products used as well as the method of analysis; harmonising sampling and analysis procedures; guaranteeing the representativeness of the samples taken (it is suggested to take 5 samples per slaughterhouse per day -taken from at least 4 different locations-, using 4 different slaughterhouses in the study, during 5 non-consecutive days, uniformly distributed over a period of 3 months; that is to say, a total of 100 samples); ensuring that all samples analysed are acceptable based on the established criteria for mesophilic aerobes (0-10 cfu/cm^2), enterobacteria (0-1 cfu/cm^2), as well as the absence/cm^2 for the pathogens *Listeria monocytogenes* and *Salmonella*.

Likewise, it is also recommended to draw up a sector-based Guide that, based on the recommendations made in this report, provides a detailed description of the protocol to be followed in order to demonstrate equivalence between the disinfection of tools in slaughterhouses and cutting rooms with hot water supplied at a temperature not less than 82 ºC and disinfection with alternative methods.

Key words

Disinfection, tools, slaughterhouses, cutting rooms.

Suggested citation

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1. Introduction

1.1 Background

Annex III of Regulation (EC) No. 853/2004 (EU, 2004) laying down specific hygiene rules for food of animal origin, establishes that slaughterhouses and cutting rooms for ungulates as well as poultry and lagomorphs "must have facilities for disinfecting tools with hot water supplied at not less than 82 ºC, or an alternative system having an equivalent effect".

On several occasions the meat sector has suggested the possibility of using alternative systems to disinfect these tools. This interest in using alternative systems is based on the claim that it would save water and energy, thereby significantly increasing the competitiveness and sustainability of meat industries.

Therefore, the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) has been requested to draft a report establishing whether disinfection with four alternative systems, under the specific conditions of use considered in the available studies, may be deemed equivalent to that conducted with water supplied at a temperature not less than 82 ºC.

In this regard, AESAN possesses information on several studies conducted by businesses in this sector on the use of various substances as alternative methods of disinfecting tools in slaughterhouses and cutting rooms.

1.2 Microbiological contamination of tools used in slaughterhouses and cutting rooms

Meat may act as a carrier of certain agents of food-borne infections and intoxications that are found in animal holdings and therefore, in living animals that arrive at slaughterhouses (Moreno, 2006). Some infectious agents that are especially noteworthy are *Salmonella* spp., *Campylobacter jejuni/coli*, enterohaemorrhagic *Escherichia coli*, *Yersinia enterocolitica* and *Listeria monocytogenes*. Meat is also a carrier of animal-associated *Staphylococcus aureus* strains (Moreno, 2006). Table 1 summarises the main hazards associated with the slaughtering and butchering of different mammals for slaughter and the severity of potential risks (Sheridan, 2004).

Table 1. Hazards associated with the slaughter of bovine, porcine and ovine animals, and the severity of potential risks (according to Sheridan (2004), adapted by Moreno (2006))

Hazard	Origin	Risk of presence	Severity
Salmonella enterica	Bovine: skin, intestine, tonsils, hooves Porcine: intestine, scalding and finishing equipment Ovis (ovine): fleece, intestine	High	Moderate or severe
Escherichia coli O157:H7	Bovine: skin, intestine, tonsils, hooves	High	Moderate or severe
Campylobacter spp.	Porcine: intestine, scalding and finishing equipment	High	Moderate or severe

Table 1. Hazards associated with the slaughter of bovine, porcine and ovine animals, and the severity of potential risks (according to Sheridan (2004), adapted by Moreno (2006))

Hazard	Origin	Risk of presence	Severity
Yersinia enterocolitica	Porcine: intestine, tonsils	High	Moderate
Listeria monocytogenes	Bovine: skin Porcine: skin, scalding and finishing equipment Ovis (ovine): fleece All species: slaughter-house facilities and equipment	High	Moderate or severe
Prions	Bovine: brain, tonsils, eyes, Central Nervous System	High	Severe
Chemical hazards*	Facilities and equipment	Low	Slight
Physical hazards	Machinery/equipment used Butchering process	Low	Slight

*Does not refer to chemical substance residue generated at the level of animal production, but to those that may be produced in the slaughterhouse itself, for example, lubricants, chlorine in the water used to wash carcasses, etc.

The different tools and utensils used in slaughterhouses and cutting rooms may lead to cross-contamination in the event that unsuitable cleaning and disinfecting procedures are used (Sanmarco et al., 1997).

Contamination of carcasses in slaughterhouses may arise from very different sources, not excluding the slaughterers themselves. Studies such as those conducted by Sanmarco et al. (1997) highlight the possible presence of *Salmonella*, and the works of O'Brien et al. (2005) and Tamplin et al. (2001) describe isolating *E. coli* in slaughterhouse carcasses.

The tools, utensils and equipment used in slaughterhouses for animals for slaughter play a very important role in the potential contamination of the carcass and the parts obtained thereof (Koutsoumanis and Sofos, 2004).

1.3 Hygiene practices for the disinfection of tools used in slaughterhouses and cutting rooms and their regulation

Different studies have been conducted to demonstrate the reduction in contamination after correct hygiene practices were applied in slaughterhouses (Rahkio and Korkeala, 1996) (McEvoy et al., 2000) (Abdalla et al., 2010).

The effect of the original natural microbiota present in animals for slaughter on the processes of alteration and the microorganisms responsible, the existing pathogens, and the control measures to be implemented, have been studied in meat and meat products (ICMSF, 1998).

Disinfecting knives in sterilisers with water at a temperature of 82 ºC is a common and compulsory

hygiene practice in slaughterhouses and cutting rooms, which has been stated in many European Union and Spanish regulations for decades.

In the European Union, different Directives and Regulations have included the need to sterilise with water at 82 ºC: Directives 64/433/EEC (EU, 1964), 92/116/EEC (EU, 1992) and 95/68/EC (EU, 1995), and Regulation (EC) No. 853/2004 (EU, 2004) currently in force.

This requirement is also mentioned in various documents and hygiene practice guides drafted by international bodies. The *Codex Alimentarius* mentions/indicates certain guidelines and also lists the specific hygiene practices in the meat sector, but in this case, it does not explicitly define a temperature, a concrete method, or a specific frequency.

Article 164 of the CAC/RCP 58/2005 (Codex Alimentarius, 2005) states that "particular cleaning programmes are required for equipment used in the slaughter and dressing of carcasses e.g., knives, saws, machine cutters, evisceration machines and flushing nozzles. Such equipment should be cleaned, and sanitised, by immersion in hot water or alternative methods, with appropriate frequency during and/or between periods of work".

Nevertheless, Section 9 (Hygiene, dressing and carcass handling) of the Manual on "Good Practices for the Meat Industry" of the Food and Agriculture Organisation of the United Nations (FAO, 2007) includes sterilisers at 82 ºC and the use of two knives by each operator (while one is used, the other is sterilised), in its list of the basic equipment required for slaughter and dressing.

To correctly disinfect the knives as well as for the purposes of good hygiene practice, it is necessary to first eliminate organic waste from the knives' surface before placing them in the steriliser. Immersing highly contaminated knives in hot water results in the coagulation of proteins on the knives' surfaces, making them inaccessible to heat or chemical disinfection. Additionally, the repeated immersion of used knives in the same hot water container may lead to fats and other organic materials accumulating on the water's surface and thus possibly re-contaminating the surface of the disinfected knife (EC, 2001).

2. Description of the systems proposed as alternatives for disinfection

Various systems have been proposed as alternatives to the use of hot water supplied at a temperature not less than 82 ºC to disinfect tools in slaughterhouses and cutting rooms.

Specifically, these systems are:

- System A, based on the use of peracetic acid diluted in osmotic tap water at room temperature.
- System B, based on the use of a peroxyacid-based compound (peracetic acid and peroxyoctanoic acid).
- System C, based on the use of hydrogen peroxide produced by electrolysis.
- System D, based on using a mixture of an alkaline detergent and a neutral non-oxidising disinfectant.

The following sections describe each of these systems as well as the studies provided to demonstrate their equivalence with the use of hot water supplied at a temperature not less than 82 ºC.

2.1 System A, based on the use of peracetic acid diluted in osmotic tap water at room temperature

It uses an oxidising solution based on peracetic acid. Peracetic acid works on the outer membrane of bacteria, bacterial endospores, yeasts and viruses, in low concentrations (0.1-0.2 %) (Block, 2001). It is considered unstable, especially when diluted, as the dilutions hydrolyse over time and lose activity (Block, 2001). It has been indicated that the by-products of its decomposition (acetic acid, oxygen and water) neither leave any residue nor pose a health hazard, thus minimising the risk to the environment and to human health (Hernández, 2006). It may be used within a wide range of temperatures (0 to 40 ºC), even in hard water, and it is effective within a pH range of 3.0 to 7.5 (Kunigk and Almeida, 2001).

As starting material, this system uses a commercial product that contains peracetic acid, which is diluted at room temperature in osmotic water (distillation-quality water obtained by reverse osmosis) to a specific concentration in sterilisers.

The peracetic acid monitoring and dosage system in question consists of an intermediate tank with a capacity greater than the total sum of litres required to fill all the sterilisers, with recirculation pumps and a product monitoring and dosage system available to ensure the required concentration of peracetic acid.

2.1.1 Studies provided with system A

The first part of the study consisted of checking the stability of the chemical product diluted in osmotic water at room temperature inside the steriliser during the work session, at a specific concentration, to obtain a minimum concentration of 250 ppm of the product inside the steriliser (Stelter, 2009). For this, a preliminary test was conducted to evaluate the stability of diluted peracetic acid in the sterilisers for a working period of 2 hours in the production line of a slaughterhouse. The peracetic acid concentration remained stable.

According to a study by Baca (2012), 100 ppm of peracetic acid is an effective disinfectant of *L. monocytogenes* and *E. coli*.

To determine the validity of peracetic acid in disinfecting knives, a series of tests were carried out to assess different aspects that may affect the effectiveness of the sterilisation.

Samples for the microbiological control of the knives' surfaces were taken with contact slides (PCA contact slides for one knife blade in order to count mesophilic aerobes -viable mesophilic aerobic microorganisms- and VRBGA contact slides on the other blade to count enterobacteria). The results obtained were considered acceptable or inacceptable according to the values considered and set by the Decision 2001/471/EEC (EU, 2001).

A prior test was conducted with the system for disinfecting tools with hot water supplied at a temperature not less than 82 ºC. The test results obtained were compared with those of the alternative system under different conditions.

2.1.2 Sterilising knives in hot water at 82 ºC

An analysis of the microbial load of the slaughterhouse knife blades was conducted throughout

the work session by immersing them in sterilisers of snout and neck skinners with hot water at 82 ºC. The counts were lower than 0.84 cfu/cm^2 in mesophilic aerobes and lower than 0.04 cfu/cm^2 in enterobacteria.

2.1.3 Sterilising knifes with the alternative system A

The evolution of the microbial load throughout the working day was then studied using sterilisers from the indicated areas (snouts and neck). In both cases, the knife was not rinsed before commencing the task of skinning the carcass.

The results showed a maximum concentration of 1.2 cfu/cm^2 of mesophilic aerobes in the snout steriliser, 0.32 cfu/cm^2 in the neck steriliser, and an enterobacterial presence lower than 0.04 cfu/cm^2 in all cases.

An additional test was conducted where the knives were rinsed with drinking water prior to sampling in order to check that possible residual compounds that might have remained on the blades of the knives used in the first tests did not influence the results. The concentration of peracetic acid was determined with special test strips, and a total absence of the chemical product was obtained in all cases. Next, it was verified that the action of peracetic acid was equally efficient, with or without rinsing prior to sampling.

The same sterilisers used in the previous test were used here. Sampling was conducted in two rounds, and in all cases, the counts were similar or lower than the test conducted without rinsing.

2.2 System B, based on the use of a peroxyacid-based compound (peracetic acid and peroxyoctanoic acid)

The product used in the study is a peroxyacid-based disinfectant (peracetic acid and peroxyoctanoic acid). Disinfection is conducted by diluting this product in water at room temperature and without rinsing after application.

Special dosing equipment is used to produce solutions with this product. The concentration is verified by conventional iodometric (redox) titration.

Peroxyacid-based disinfectants are used to decontaminate carcasses, especially bird carcasses. Decontaminating with this or other products is an option used by slaughterhouses in some countries (for example, United States and Canada) for better hygienic-sanitary quality and delayed alteration of the products. In other countries, such as European Union states, it is currently prohibited for the decontamination of carcasses, except in the case of beef using certain concentrations of lactic acid (EU, 2013).

2.2.1 Studies provided with system B

It is a study presented during a scientific meeting (Heres and Verkaar, 2011). The study was conducted with the goal of ascertaining its effectiveness in sterilising knives used in a pork slaughterhouse in the Netherlands. The reference method was the use of a steriliser with water at 82 ºC. The microbial groups studied were counts of mesophilic aerobes and *Enterobacteriaceae*.

The knives were submerged in hot water at 82 ºC or in the disinfectant for 0, 1, 10, 30 and 60 seconds, respectively. The knife blades were sampled with Rodac plates (PCA for counting mesophilic aerobes -total viable- and VRBGA for enterobacteria).

The disinfecting effect of water at 82 ºC and that of the peroxyacid-based disinfecting solution were assessed for 3 days. Knives from 5 different positions in the slaughter line were disinfected in conventional knife sterilisers. Samples were taken from both sides of the blade: without disinfecting, after 1 second and after 1 minute.

The results show that after 1 minute of exposure in both methods, the effectiveness of the peroxyacid-based disinfectant was equal to or higher than water at 82 ºC for both mesophilic aerobes and for enterobacteria in the sampled surfaces, in all cases. A total of 60 samples were taken after evisceration and 60 after the Achilles tendon incision.

2.3 System C, based on hydrogen peroxide produced by electrolysis

Hydrogen peroxide (H_2O_2) is a powerful oxidant widely used as a disinfectant. The system is based on a catalytic electrolysis process that is installed in-line, in the pipes distributing the water.

Documentation related to different trials, reports, validations in certain industry sectors, studies and examples of use have been provided to demonstrate the effectiveness of the technology.

2.3.1 Studies provided with System C

The study has been conducted exclusively in the cutting room of a slaughterhouse for Iberian pork, and not in the slaughter-dressing bay or room.

The goal of the study was to verify the effectiveness of hydrogen peroxide in cleaning and disinfecting knives in slaughterhouses with a high contaminant load and to validate this technology as a substitute for the traditional method employing water at 82 ºC.

The study was based on a combination of two protocols:

- Standard UNE-EN 13697:2015+A1:2020 (UNE-EN, 2020) (Chemical antiseptics and disinfectants). Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. Test method and requirements without mechanical action (phase 2/step 2). Alternative bacteria have been used instead of those set by the standard.
- The Protocol of the Department of Agriculture, Food and the Marine of Ireland, titled "Alternative systems for disinfecting tools in meat plants" (Department of Agriculture, Food and the Marine of Ireland, 2012). The cutting tools are disinfected only with the alternative system. The left side of the cutting tool is swabbed immediately after use and prior to disinfection. The right side of the cutting tool is swabbed immediately after disinfection. The time of disinfection of the cutting tool using the alternative system must be logged for each swab.

Following the Irish protocol, 4 rounds of sampling were conducted on the 4 knives.

For each round, 4 samples of meat were prepared, contaminated with 1 ml of each suspension and left to rest for 1 minute, subsequently several cuts were made in each piece, imitating the use

of the knife in normal activity. Next, a sample was taken by swabbing the left side of the entire knife blade, and the knife was then immediately placed in the recipient with hydrogen peroxide. After 15 minutes of disinfection, the knife was taken out and a sample was taken from the right side of the entire knife blade with a new swab. All the samples were kept refrigerated at temperatures between 4 and 8 ºC until processed at the laboratory.

The following microbiological tests were conducted (the culture medium is indicated):

- PCA for mesophiles at 30 ºC (mesophilic aerobes).
- VRBGA for enterobacteria (*E. coli*).
- ChromoSalm for *Salmonella*.
- ALOA for *Listeria*.

The results obtained showed that the samples after disinfection had counts lower than 10 cfu/cm^2 and were undetectable in most analyses, for all microorganisms (mesophilic aerobes, enterobacteria, *Salmonella enterica* and *L. monocytogenes*).

2.4 System D, based on a mixture of an alkaline detergent and a neutral non-oxidising disinfectant

It is an alkaline detergent based on sodium hydroxide and sodium hypochlorite combined with a neutral non-oxidising disinfectant which contains, among other compounds, N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine and salicylic acid.

The combined product may be used in CIP (cleaning *in situ* processes) either alone or in an acidic or alkaline solution, and it may also be used by spraying or immersion.

2.4.1 Studies provided with system D

The test was conducted on a cylindrical disinfection system with capacity for 8 knives and 3 knives were used. The 3 knives were coded by the hilt colour (red, black and grey). 2 tests were conducted.

The first test was conducted with detergent added to drinking water (3 g/l) and samples of the knife surfaces were taken after 2 and 4 hours of work (the knives were rinsed with drinking water before being placed in the disinfecting container). The presence of aerobes was detected at lower than 10 cfu/cm^2 except in one sample >10^2 cfu/cm^2. The enterobacteria counts were lower than 5 cfu/cm^2 and *Listeria* was not detected.

The second test was conducted with 6 g/l of detergent based on sodium hydroxide and sodium hypochlorite and 3 g/l of neutral non-oxidising disinfectant, and samples of the knives' surfaces were taken after 4 and 8 hours of work (the knives were rinsed with drinking water before being placed in the disinfecting container). The presence of aerobes was detected at under 10 cfu/cm^2, and enterobacteria and *Listeria* were absent.

Finally, a third test was conducted after 8 hours of work (to study what might happen in the event that the work session was extended). The presence of aerobes was detected at under 5 cfu/cm^2 and enterobacteria and *Listeria* were absent.

3. Comments on the studies reviewed

The studies presented constitute a notable effort to demonstrate the equivalence between the disinfection of tools in slaughterhouses and cutting rooms with hot water supplied at a temperature not less than 82 ºC, and other alternative systems of disinfection.

However, these studies display various degrees of methodological deficiencies that may be summed up as:

- Methods of analysis: the method of microbiological analysis (contact slides) is not suitable for conducting these studies, a more suitable method is to use swabs.
- Microorganisms: they do not include the analysis of foodborne pathogenic microorganisms of interest in slaughterhouses. Mesophilic aerobes and enterobacteria are studied. *Salmonella* is only included in one study and *Listeria* in two.
- Number of samples and establishments: the number of sampling days or analysis segments and slaughterhouses is insufficient.
- Information on the products and protocols used: the information on the disinfecting products used is insufficient. The studies conducted and the method of analysis used are not described in detail.

4. Sampling recommendations

In order to demonstrate the acceptability of alternatives to sterilisation with water at a minimum temperature of 82 ºC, it is necessary to follow a sampling procedure established for this purpose. In this regard, it must fulfil the following requirements:

- Independence of the samples taken in the slaughterhouse. For this, the samples shall be taken within the same time interval, from different sterilisers.
- Harmonising sampling and testing procedures, so that the results may be compared.
- Representativeness of the samples taken, mainly in different locations within a single slaughterhouse, as well as in different slaughterhouses.
- It is assumed that all the samples analysed must be acceptable according to the criteria established for mesophilic aerobes (0-10 cfu/cm^2), enterobacteria (0-1 cfu/cm^2), as well as the absence/cm^2 for the pathogens *L. monocytogenes* and *Salmonella*.

Given that the nature of a sampling plan is associated with a statistical foundation, it is necessary to assume a starting hypothesis and a confidence level in order to determine the number of samples to be taken.

Statistical distributions are used to represent the frequency or probability of the appearance of a set of values. In order to determine the minimum number of samples to be taken for the acceptability of the alternative methods studied, a Binomial distribution has been used as well as *a priori* Beta distribution. The Beta distribution is defined by two parameters; $\alpha = s+1$; and $\beta = n-s+1$; where s= number of positive or unacceptable samples; and n= total number of samples.

Binomial distribution is in turn defined by the parameters n= total number of samples; and p= probability of success or detection of positive samples. In this case, a combined Beta-Binomial distribution

has been used to estimate the number of positive samples (y): Binom (n, p), where p= Beta (α, β).

In this regard, the % of positives/1000 samples has been estimated by applying different sampling plans within a range n= 10-200; c= 0.

% positives= y/n

% positives/1000 samples= (y/n)*1000

From the generated data, a Monte Carlo simulation is performed in @Risk with 1000 iterations, obtaining a series of probability distributions that represent the frequency of appearance of positives/1000 samples.

To estimate the minimum number of samples to be taken, the mean value obtained from each distribution of the number of positives/1000 samples has been related with the values n= 10; 50; 100; 150 and 200 (Figure 1).

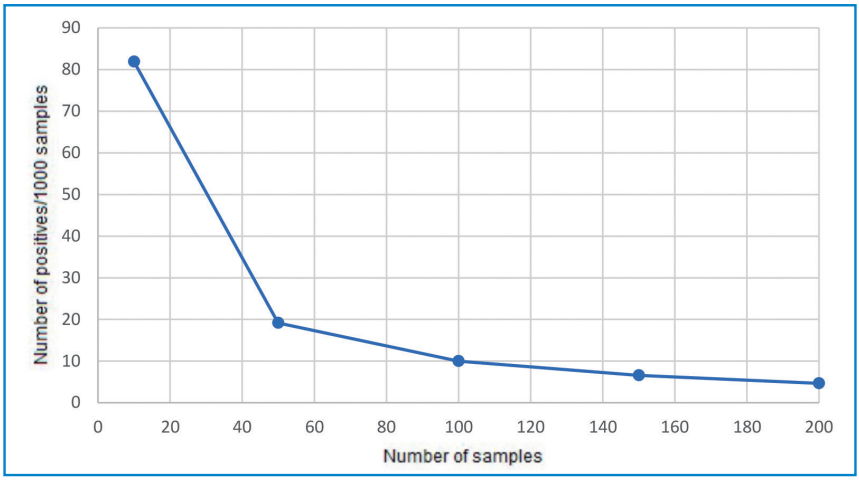

Figure 1. Graphical representation of the relationship between the estimated value of the number of positives/1000 samples and the number of samples taken, assuming a value of c= 0.

As may be seen in Figure 1, the fact of taking a number higher than 100 samples does not have a significant effect on the decrease in the number of positives/1000 samples, and therefore the selection of sampling plan n= 100; c= 0 is considered appropriate.

Finally, and in order to increase representativeness, to consider the acceptability of alternative methods, it is suggested to take 5 samples per slaughterhouse and per day (taken from at least 4 different locations), using 4 different slaughterhouses, during 5 non-consecutive days uniformly distributed over a period of 3 months (100 samples in total).

Conclusions of the Scientific Committee

Sterilising knives with hot water at a temperature not less than 82 ºC is a way to guarantee adequate food safety in slaughtering and cutting operations.

Various studies on alternative systems have been reviewed where it is observed that the compounds used lead to reductions in the studied microorganisms that contaminate tools used in slaughterhouses and cutting rooms. The results display reductions similar to those achieved by official methods in the conditions of these studies.

However, these studies have methodological limitations (number of repetitions, sampling plan, microorganisms used, and method of analysis) which prevents us from establishing whether they are equivalent to the currently approved method for disinfecting tools (in hot water at a temperature not less than 82 ºC).

It is recommended to draw up a sector-based Guide that, following the recommendations made in this report, provides a detailed description of the protocol to be followed in order to demonstrate equivalence between the disinfection of tools in slaughterhouses and cutting rooms with hot water supplied at not less than 82 ºC, and disinfection with these and other substances.

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