International Journal of Dairy Technology

SDT Society of Dairy Technology

doi: 10.1111/1471-0307.12516

REVIEW

A review of test protocols for the evaluation of teat disinfectants

SARAH FITZPATRICK,[1,2] MARY GARVEY[2] and DAVID GLEESON[1,*]

[1]*Teagasc Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, County Cork, Ireland, and*
[2]*Cellular Health and Toxicology Research Group, Institute of Technology Sligo, County Sligo, Ireland*

The prevention of mastitis is the most important part of a mastitis control programme. Postmilking disinfection is considered the most effective procedure for preventing new intramammary infections in dairy herds. This article reviews the different protocols used to evaluate the efficacy of teat disinfectant products. These protocols include experimental challenge, natural exposure and excised teat, along with agar diffusion assays and teat swabbing. The experimental designs, limitations, positive and negative aspects of each protocol were compared and discussed throughout this article.

Keywords Teat disinfectants, Product efficacy, Experimental challenge, Natural exposure, Excised teat, Mastitis.

INTRODUCTION

Bovine mastitis is an inflammation of the mammary glands within the udder and classified as intramammary infections (IMIs) (Bradley 2002). Mastitis can be further classified as contagious or environmental. Contagious bacteria can adapt to survive within the host (mammary glands of the udder), establish subclinical infections and can be spread from cow to cow during milking (Radostits *et al.* 1994). Environmental bacteria are opportunistic invaders of the udder but cannot adapt to live within the host. They usually cause an immune response and are quickly removed (Bradley 2002).

Teat disinfectants have been used to reduce the number of new IMIs caused by mastitis-causing pathogens (Schmidt *et al.* 1984; Foret *et al.* 2005). This practice is widely accepted as a fundamental part of a successful mastitis control programme (Oliver *et al.* 1991; Oura *et al.* 2002; Leslie *et al.* 2006). Teat disinfectants may be used pre- and/or postmilking. Premilking disinfection involves application by dipping or spraying teats in disinfectant, followed by wiping off the disinfectant solution approximately 30 s after application and prior to milking (Gleeson *et al.* 2009). Premilking teat disinfection has been found to be more effective in controlling environmental bacteria such as *Escherichia coli* and *Streptococcus uberis* (Enger *et al.* 2015, 2016) with new IMIs 48% lower compared to postmilking disinfection only (Oliver *et al.* 1993). Postmilking disinfection involves applying a disinfectant to the teat directly after the milking clusters have been removed, and has been found to assist in the reduction in mastitis caused by environmental and contagious bacteria, such as *Streptococcus agalactiae and Staphylococcus aureus*, by 53%, compared to undisinfected teats (Wesen and Schultz 1970).

There are several different active ingredients found in products used for teat disinfection. The main disinfectant ingredients incorporated in products are iodine, chlorhexidine gluconate, chlorine dioxide and lactic acid, with many combinations of these ingredients. There are many different types of teat disinfectant products such as barrier teat disinfectants, powders, teat foams, high viscosity liquids, liquid concentrates and ready-to-use liquid disinfectants. Teat disinfectant products must meet many different criteria to serve their purpose in modern milking parlours (Godden *et al.* 2016). They must be persistent, have proven germicidal efficacy, prevent new IMIs, maintain optimal teat condition and aid in the healing of teat skin as this is the main, and natural, defence barrier against infection (Lago *et al.* 2016). Cracked skin is more

*Author for correspondence. E-mail: David.Gleeson@teagasc.ie

likely to harbour mastitis-causing bacteria (Blowey and Edmondson 2010; Lago *et al.* 2016). Disinfectants must not leave any harmful residues in milk that could affect public health (Godden *et al.* 2016). For example, the application of an iodine as a premilking disinfectant can have implications for processors as unacceptable levels of iodine can occur in the milk (O'Brien *et al.* 2013).

For products to be considered satisfactory, they must be compliant with at least the minimum standards of efficacy (Boddie *et al.* 2004). The European standards (such as EN 1656 and EN 1276) are generally used to determine the threshold of efficacy for different disinfectants. The purpose of these tests is to evaluate the bactericidal, fungicidal, yeasticidal, basic sporicidal or mycobactericidal activity of products used under various conditions (ATS, 2014). Many tests are suspension based and used to support general antimicrobial claims, while other tests are carrier based and used to support the antimicrobial activity of products on surfaces for devices. Any disinfectant or antiseptic chemicals intended for registration within Europe are tested using a variety of methods developed by the CEN (European Committee for Standardisation; Ats 2014). Interpretation of the results of these methods is very important. According to Boddie *et al.* (2002), an effective teat disinfectant should give a minimum yield of 3 log reduction, with 4–5 log reduction being preferable, for treated teats, when 6 log units are recovered from untreated control teats, all within 10 min of application. This reduction has been considered an acceptable estimate of germicidal activity when using the excised teat protocol (Schmidt *et al.* 1984; Watts *et al.* 1984). In comparison, EN 1276 requires a 5 log reduction within 60 min of application.

Protocols determining the effectiveness of teat disinfection products in controlling mastitis

The National Mastitis Council (NMC), based in Minnesota, USA, monitors the development of protocols that determine the effectiveness of mastitis control products such as teat disinfectant solutions (Hogan *et al.* 1990). Due to the lack of uniformity between governmental standards of teat disinfectant products (Schukken *et al.* 2013), the NMC research committee and the Teat Dip committee reviewed and updated guidelines and protocols in an effort to standardise procedures to ensure uniform and accurate comparison of studies (Schukken *et al.* 2013). These guidelines include (1) challenge organisms for experimental exposure studies; (2) procedures for statistical analyses; (3) appropriate sample size to detect a difference between treatments; (4) milking systems operations and udder preparation procedures; and (5) removal of the requirements for at least two herds and a study length of at least 12 months from the natural exposure protocol (Nickerson *et al.* 2004).

The methods discussed by the NMC include the natural exposure, the experimental challenge and the excised teat protocols (Nickerson 2001; Enger *et al.* 2015). Agar diffusion (disc diffusion or well diffusion) assays have been used in many clinical microbiological laboratories for antimicrobial susceptibility testing (Balouiri *et al.* 2015). This assay has not been as extensively used as the other protocols, but it could be adapted to determine the efficacy of teat disinfectant products.

The objective of this review is to evaluate the various methodologies used to determine the efficacy of teat disinfectants. These methods can include *in vitro* (standard laboratory) and *in vivo* (in-field) tests (Godden *et al.* 2016). *In vivo* testing refers to experimentation using a whole, living organism (i.e. herd of cows) within its natural environment as opposed to a living or dead organism (i.e. excised teat) in a laboratory. These tests can include teat swabbing, an experimental challenge and natural exposure protocols. This review also discusses the experimental design such as sampling procedures, split herd and udder designs between different studies. *In vitro* testing refers to performing a procedure in a controlled environment (laboratory), outside of a living organism. These tests can include excised teat protocol and agar diffusion assay. A number of reviewed studies were compared to determine differences between the experimental design applied and the results obtained (Table 1). The limitations and positive and negative aspects of each protocol have also been discussed in this review.

IN VIVO TESTING

In vivo testing has benefits over laboratory tests as it observes the overall effects of a disinfectant product within the environment. This can help to determine the efficacy of products against new IMIs in actual dairy herds as opposed to identifying the efficacy of a disinfectant product within a laboratory situation.

Experimental challenge protocol

The experimental challenge protocol is commonly applied on a research farm and is the industry standard for manufacturers of agricultural disinfectants for determining the efficacy of new and existing teat disinfectant products. The aim is to reduce the incidence of new IMIs compared with non-disinfected controls when teats are challenged with mastitis-causing bacteria to increase the infection rate (Nickerson 2001). Directly after milking, cows' teats are dipped with a suspension of bacteria, and immediately afterwards, two diagonally (left front, right rear) or horizontally (front left, front right) opposite teats are disinfected in the test product and the remaining teats are either left not disinfected to serve as negative controls or disinfected with a product of known efficacy to serve as a positive control (Nickerson *et al.* 2004). Milk samples may be taken weekly for a number of weeks from each intramammary quarter and tested to determine the number of new infections arising from the bacterial suspension. The disinfected and control quarters are then compared, and efficacy is expressed as the

Table 1 Summary of the research protocols included in this review.

Study	*Teat swabbing*	*Excised teat*	*Natural exposure*	*Experimental challenge*	*Split udder*	*Split herd*	*Control*	*Result*
Enger *et al.* (2015)	✗	✓	✗	✗	N/A	N/A	Negative	% Reduction: Contagious: 88%, Enviro.: 82%, CNS: 80.0%
Boddie *et al.* (2002)	✗	✓	✗	✗	N/A	N/A	Negative	LR of 6.29 (*M. bovigenitalium)*, 5.41 (*M. bovis*) & 5.70 (*M. californicum*)
Schmidt *et al.* (1984)	✗	✓	✗	✗	N/A	N/A	Neg. & Pos.	LR of 78.5–100% for Gram-negative bacteria
Philpot *et al.* (1978)	✗	✓	✗	✗	N/A	N/A	Neg. & Pos.	Iodophor, sodium hypochlorite & sodium dichloro-s-triazenetrione: effective against all strains
Watts *et al.* (1984)	✗	✓	✗	✗	N/A	N/A	Neg. & Pos.	LR of 3–4 for *Staph. aureus* & *Str. agalactiae*
Lago *et al.* (2016)	✗	✗	✓ 12 weeks	✗	✗	✓	Positive	EX was 17% more effective than the PC
Godden *et al.* (2016)	✗	✗	✓ 12 weeks	✗	✗	✗	Positive	Product found to be noninferior
Foret *et al.* (2005)	✗	✗	✓ 9 months	✗	✓	✗	Positive	Major pathogens reduced by 58% & minor pathogens by 54%
Ceballos-Marquez *et al.* (2013)	✗	✗	✓ 10 weeks	✗	✗	✗	Positive	EX was 19% more effective than the PC
Oliver *et al.* (1991)	✗	✗	✓ 11&12 months	✗	✗	✗	Negative	Efficacy against minor pathogens (35%), CNS (33.1%) & *C. bovis* (37.8%)
Boddie *et al.* (2004)	✗	✗	✗	✓ 8 weeks	✓	✗	Negative	% reduction: 88% (*Staph. aureus)* & 67% (*Str. agalactiae)*
Leslie *et al.* (2006)	✗	✗	✗	✓ 10 weeks	✓	✗	Positive	No differences found between products
Oura *et al.* (2002)	✗	✗	✗	✓ 12–14 weeks	✓	✗	Negative	Reduction of 72% & 75% and 100% & 88% for *Staph. aureus* & *Str. agalactiae,* respectively
Foret *et al.* (2003)	✗	✗	✗	✓ 7 weeks	✓	✓	Positive	% reduction: 90% (*Staph. aureus)* & 73% (*Str. agalactiae*)
Leslie *et al.* (2005)	✗	✗	✗	✓ 10 weeks	✓	✓	Positive	No differences found between EX & PC
Gleeson *et al.* (2009)	✓	✗	✗	✗	N/A	N/A	N/A	Teat disinfectants had different efficacies when used on cows indoors or outdoors
Gibson *et al.* (2008)	✓	✗	✗	✗	N/A	N/A	N/A	No significant difference between treatments in the reduction in TVC
Baumberger *et al.* (2016)	✓	✗	✗	✗	N/A	N/A	N/A	No differences in reduction between treatment for most bacterial counts
Mišeikienė *et al.* (2015)	✓	✗	✗	✗	N/A	N/A	N/A	Lactic acid and iodine had highest probability of reducing TBC

CNS, Coagulase Negative Staphylococci; EX, experimental control; *M. bovigenitalium, Mycoplasma bovigenitalium; M. bovis, Mycoplasma bovis; M. californicum, Mycoplasma californicum; C. bovis, Corynebacterium bovis*; LR, log reduction; PC, positive control; TBC, total bacterial contamination; TVC, total viable count.

percentage reduction in new infections in disinfected quarters (Nickerson 2001).

Bacteriological status of herd

Bacteriological status of the herd refers to the determination of the infection status of each cow involved in the study. This status gives a baseline for determining the rate of new IMIs during the study. Studies reviewed in this article carried out an initial bacteriological infection status for the herd before the start of each study. Of the five studies reviewed, Boddie *et al.* (2004) and Leslie *et al.* (2005) have longer periods of bacteriological records for their herds. Leslie

et al. (2005) established the bacteriological status by sampling the cows weekly for 3 weeks prior to the study. Boddie *et al.* (2004) determined the bacteriological status of the herd prior to the study. The herd was sampled monthly throughout the year so that all cows had lifetime cultures for all lactations. When comparing the bacteriological results at the end of the study to the initial herd status, either a 3-week bacteriological status study or a lifetime culture record would give a satisfactory outlook on the actual bacteriological status of the overall herd. This information can be used to resolve discrepancies in infection results, should they occur during the study.

Collecting milk samples and definition of new IMIs

Within the reviewed studies, intramammary quarter milk samples were collected on a weekly basis and each teat forestripped before a sample was collected. Foret *et al.* (2003) and Boddie *et al.* (2004) additionally used a cotton pledget moistened with 70% w/v alcohol to wipe each teat, which is somewhat similar to the Animal Health Ireland (AHI) CellCheck recommendations (CellCheck, 2012). Milk samples were then stored at 5 °C (Foret *et al.* 2003; Boddie *et al.* 2004) and −20 °C (Oura *et al.* 2002). When defining a new IMI, Oura *et al.* (2002), Foret *et al.* (2003) and Boddie *et al.* (2004) had similar standards, such as (1) *Staph. aureus* or *Str. agalactiae* was isolated from a clinical intramammary quarter, (2) two consecutive samples yielded >500 cfu/mL of the same pathogen, or (3) three consecutive samples contained 100–400 cfu/mL of the same pathogen. Oura *et al.* (2002) had an additional fourth standard where five consecutive samples contained >1 cfu/mL of the same pathogen were considered a new IMI. This was different to Leslie *et al.* (2005, 2006), who confirmed a quarter as positive for new IMI if any of the following criteria were met: (1) signs of clinical mastitis were observed (samples were taken to confirm bacteria present), (2) *Staph. aureus* or *Str. agalactiae* were cultured in high numbers (>500 cfu/mL) in two consecutive weeks, and (3) *Staph. aureus*, *Str. agalactiae*, *Staphylococcus* species or *Corynebacterium bovis* were cultured in low numbers (100–500 cfu/mL) in three consecutive samples. In addition to these criteria, somatic cell count (SCC) data were used to screen culture negative intramammary quarters or to show elevated SCC. Any quarter with a SCC of >300 000 cells/mL were resampled and submitted for culture. These differences in criteria used to define new IMIs make comparing results from different studies challenging. The criteria described by Foret *et al.* (2003) and Boddie *et al.* (2004) would be preferred by the authors as the most suitable criteria for defining new IMIs.

Challenge suspension and application

All reviewed studies created stock suspensions with a concentration of 5×10^7 cfu/mL for each bacterial strain, before application to the teats. Differences in the preparations of stock suspension were observed between the different studies. Leslie *et al.* (2005, 2006) created *Staph. aureus* and *Str. agalactiae* stock suspensions from culture stocks, three times weekly. Foret *et al.* (2003) and Boddie *et al.* (2004) prepared stock suspensions from lyophilised vials, of *Staph. aureus* weekly and suspensions of *Str. agalactiae* were prepared daily. Oura *et al.* (2002) used isolated colonies of *Staph. aureus* and *Str. agalactiae* and created both stock suspensions daily. The creation of stock solutions daily may help to reduce the chances of the stock solution becoming contaminated. In all studies, the suspensions were applied to all four teats immediately after removing the milking cluster and the suspension left to dry before the application of teat disinfectant. However, differences in application schedules were observed between studies. Oura *et al.* (2002) applied the suspension for 7 days each week after the morning milking and Leslie *et al.* (2005, 2006) applied the suspension for 7 days each week, but after morning and afternoon milking. Studies by Foret *et al.* (2003) and Boddie *et al.* (2004) applied the suspension for 5 days each week after the afternoon milking. The reduced challenge days may have an impact on the outcome of results as the challenge exposure time was reduced.

Experimental design

Studies undertaken by Oura *et al.* (2002), Foret *et al.* (2003), Boddie *et al.* (2004) and Leslie *et al.* (2005, 2006), used varying herd numbers ranging from 45 to 165 cows and also varying trial periods from a 7- to 14-week period. All studies were satisfied that the herd numbers enrolled in the studies accounted for possible errors and gave representable results with the exemption of Leslie *et al.* (2006) who stated that the low animal number used (45 cows) may have limited the possibility of detecting differences between treatment groups. This would indicate that a herd size lower than 90 cows may be insufficient in an experimental challenge study.

All reviewed studies are in agreement with using the split udder design when applying the teat disinfectant, with Foret *et al.* (2003) and Leslie *et al.* (2005) also implementing a split herd design. Oura *et al.* (2002), Foret *et al.* (2003) and Boddie *et al.* (2004) used negative controls by leaving the other half of the udder as undisinfected controls. This is less likely to be carried out in future studies as it can be seen as unethical due to leaving teats exposed to an increased level of IMIs (Schukken *et al.* 2013). Studies by Leslie *et al.* (2005, 2006) used a positive control teat disinfectant, which may be more favourable in terms of animal welfare as the teats are still given protection against the introduced bacteria.

Evaluation of disinfectant products using 'experimental challenge protocol'

In a number of challenge studies, different bacterial reductions were observed when different disinfectant products

were evaluated. Studies by Foret *et al.* (2003), Boddie *et al.* (2004) and Leslie *et al.* (2005) evaluated iodine disinfectant products. Boddie *et al.* (2004) found that a 0.1% w/w iodine teat disinfectant reduced the infection rate for *Staph. aureus* by 88% and 67% for *Str. agalactiae*, whereas Foret *et al.* (2003) found that a 1% w/w iodine concentration reduced the infection rate by 90% for *Staph. aureus* and 73% for *Str. agalactiae*. Leslie *et al.* (2005) compared a 1% w/w iodophor product against a commercially available product and found there were no significant differences in new IMIs caused by *Staph. aureus* and *Str. agalactiae*. Leslie *et al.* (2006) also evaluated disinfectants containing hydrogen peroxide (H_2O_2) and dodecylbenzene sulphonic acid (DBSA) against a commercially available product. The authors concluded that there was no significant difference between the two products in new IMIs caused by *Staph. aureus* and *Str. agalactiae*. A further study by Oura *et al.* (2002) compared two different concentrations of dodecylbenzene sulphonic acid (DBSA) (0.53% w/w vs 0.27% w/w). They found new IMIs were reduced by 72% (*Staph. aureus)* and 75% (*Str. agalactiae)* and 100% (*Staph. aureus)* and 88% (*Str. agalactiae*), respectively.

Summary

Use of the experimental challenge protocol allows the tester to evaluate the effectiveness of the teat disinfectant but may not represent the efficacy of the product in the chosen environment as only the bacterial suspension bacteria are of interest, while the new IMI could be caused by any other mastitis-causing bacteria. The design of the experiment must be efficiently planned to avoid any animal health welfare issues (Schukken *et al.* 2013) and, as stated by Nickerson *et al.* (2004), the experimental challenge protocol must be carried out in research farms. The increased infection status of the herd as a result of challenge application would prevent the use of this protocol on commercial farms. The experimental designs used in experimental challenge studies can be compared to other studies to enable comparison of results. In comparison with a natural exposure protocol, a challenge study can be carried out in a shorter time period but involves a higher workload in the preparation of the challenge suspensions, such as ensuring suspensions are free from contamination, the application of the challenge suspension, drying and then the application of the teat disinfectant. As the teats of the cow are challenged with bacteria, an increased rate of new IMIs would be expected as compared to natural exposure studies. Therefore, the use of controls is needed within the protocol to ensure correct reductions are determined.

Although the experimental challenge protocol evaluates the product within the environment, it may not accurately simulate the efficacy of a product as the infection is not naturally occurring. Furthermore, the increased infection status and rate of new IMIs within the herd, due to the introduction of challenge bacteria, would not be representative of the situation on commercial farms.

Natural exposure protocol

The natural exposure protocol is suitable for application on commercial herds and can be used to evaluate the efficacy of a teat disinfectant product in reducing the incidence of new IMIs, without the requirement of challenging teats with mastitis-causing bacteria. The new infection rate is dependent on natural exposure to mastitis-causing bacteria in the farm environment of the cow. In natural exposure studies, other protocols such as noninferiority studies, split herd and split udder models have been used (Nickerson 2001). Noninferiority studies compare a teat disinfectant of unknown efficacy to a teat disinfectant of known efficacy (also referred to as positive control studies; Godden *et al.* 2016). Intramammary quarter milk samples are taken from nondisinfected and disinfected teats of each cow and results compared against each other to determine the rate of reduction in IMIs (Nickerson 2001). A split herd refers to disinfecting one half of the herd and leaving the other half of the herd without disinfection or a known disinfectant used as a control. Somatic cell count results are compared between herds to determine the rate of reduction in IMIs (Nickerson 2001). A split udder model refers to half the teats of the cow being disinfected, with the other half left undisinfected (negative control) or a control disinfectant is applied (positive control). A split udder protocol is commonly used in natural exposure studies (Nickerson *et al.* 2004).

There were many differences between the numbers of cows enrolled in the reviewed studies and in the length of the study periods. Oliver *et al.* (1991) had the highest number of cows enrolled in their study with 374 cows, followed by Lago *et al.* (2016) and Godden *et al.* (2016) with 299 and 317 cows, respectively. Foret *et al.* (2005) and Ceballos-Marquez *et al.* (2013) used 90 and 199 cows, respectively. Oliver *et al.* (1991) and Foret *et al.* (2005) undertook their studies for the longest period of 9–12 months. In comparison, Ceballos-Marquez *et al.* (2013), Godden *et al.* (2016) and Lago *et al.* (2016) carried out their studies within a 10- to 12-week period. The sample size and study length used in the studies gave adequate statistical power when analysing results.

Bacteriological status of the herd and sampling schedule

A review of studies by Oliver *et al.* (1991), Foret *et al.* (2005), Ceballos-Marquez *et al.* (2013) and Godden *et al.* (2016) showed that the bacteriological status of their herds was determined by collecting milk samples before the commencement of the study. These samples were used to determine the infection status and create a baseline of each individual cow. Within the reviewed studies, intramammary quarter milk samples were collected. Oliver *et al.* (1991), Foret *et al.* (2005), Godden *et al.* (2016) and Lago *et al.*

(2016) collected milk samples every 2 weeks with Foret *et al.* (2005) collecting a monthly sample, whereas Ceballos-Marquez *et al.* (2013) collected milk samples biweekly but only cultured them depending on SCC analysis.

Analysis of milk samples and definition of new IMIs

A review of studies conducted by Ceballos-Marquez *et al.* (2013), Godden *et al.* (2016) and Lago *et al.* (2016) used SCC analysis to decide whether a sample should be cultured and considered samples from clinical cases as eligible for identifying new IMIs. Godden *et al.* (2016) and Lago *et al.* (2016) determined subclinical mastitis using SCC levels above a defined threshold and an IMI as being present when a single colony from a sample was isolated from any bacterial species with the exception of coagulase-negative *Staphylococcus* (CNS) and *Bacillus* species. A single IMI was defined as the presence of only one pathogen type in the sample, whereas mixed infections corresponded to the presence of two different bacterial species. In comparison, Oliver *et al.* (1991), Foret *et al.* (2005) and Ceballos-Marquez *et al.* (2013) considered an IMI as a new infection based on the presence of a microorganism that was not identified in that quarter previously. Ceballos-Marquez *et al.* (2013) used single intramammary quarter samples to determine new IMIs while Oliver *et al.* (1991) used duplicate individual quarter milk samples and Foret *et al.* (2005) used two consecutive quarter milk samples to determine new IMIs.

To standardise the routine identification of new IMI from quarters not showing signs of clinical infection, quarters with a SCC >300 000 cells/mL and with a pathogen present on one occasion could be considered a new IMI.

Split herd vs split udder

When considering whether to use split herd or split udder sampling, split herd appears to give a more representative result of product efficacy (Enger *et al.* 2016; Lago *et al.* 2016). Although, with this in mind, Enger *et al.* (2016) state that future natural exposure studies using a nondisinfected negative control treatment should employ a split udder design rather than a split herd design to help to reduce the effect of a teat disinfectant efficacy study on herd udder health. Furthermore, Lago *et al.* (2016) state there are some advantages to using a split herd design rather than a split udder design. This is due to the split herd design remaining more realistic when using a large number of animals. It is also unlikely to affect the prevention and treatment methods used at cow level due to the interdependence between intramammary quarters.

Evaluation of disinfectant products using 'natural exposure protocol'

Many studies have evaluated the efficacy of a range of different iodine concentrations in disinfectants. A study by Oliver *et al.* (1991) evaluated a postmilking teat disinfectant that contained 0.25% w/w available iodine, glycerine and water. In a herd with a high prevalence of contagious mastitis, the overall efficacy of the experimental product against mastitis pathogens was 62%. In a herd with a high prevalence of environmental pathogens, the overall efficacy of the product against major mastitis pathogens was 24%. Similarly, Foret *et al.* (2005) evaluated the application of 0.25% w/w iodine postmilking teat disinfectant. The product reduced the infection rate for major pathogens by 58% and minor pathogens by 54%. Similarly, Ceballos-Marquez *et al.* (2013) found that the application of a premilking 0.5% w/w iodine teat disinfectant was 19% more effective compared to the positive control. While Foret *et al.* (2005) used a low concentration (0.25% w/w) iodine-based product as a postmilking disinfectant, it was more effective than a higher concentration (0.5% w/w) iodine-based product used by Ceballos-Marquez *et al.* (2013) as a premilking disinfectant.

The evaluation of a new range of active ingredients in teat disinfectant products in comparison with iodine-based products is beneficial due to the potential iodine residues in milk which could possibly affect human health (O'Brien *et al.* 2013). Studies by Godden *et al.* (2016) and Lago *et al.* (2016) evaluated a glycolic acid-based postmilking teat disinfectant. Both studies concluded that the experimental product was more effective at reducing IMIs than a positive control.

Summary

The natural exposure protocol can give a better indication of how the teat disinfectant would work within the environment. A limitation of the natural exposure protocol is that it can be time-consuming with studies normally being conducted during a full lactation to get a complete comparison between disinfected and nondisinfected controls. However, when testing the efficacy of teat disinfectants, the number of diagnostics needed to determine a new IMI, along with a large number of bacteriological culturing can raise costs and leave the protocol unreasonably expensive (Ceballos-Marquez *et al.* 2013). Ceballos-Marquez *et al.* (2013) addressed this limitation by evaluating the use of a novel two-step diagnostic process to evaluate a premilking disinfectant. Rather than conducting the study over a period of 10–12 months, they used a 10-week period. In this study, authors used SCC analysis to determine whether samples should be cultured. This resulted in a more economical and efficient way to identify new IMIs. This process was also used by Godden *et al.* (2016) and Lago *et al.* (2016) during a postmilking efficacy trial. Another possible limitation is the use of study herds with different levels and types of infection. As stated by Oliver *et al.* (1991), many studies have been conducted on herds with a high prevalence of contagious mastitis which can make the evaluation of teat disinfectant efficacy against environmental pathogens difficult. Therefore, efficacy studies should be conducted on two separate herds with one having a high prevalence of

contagious and the other with a high prevalence of environmental pathogens to generate the number of IMI necessary for determining the efficacy of the disinfectant.

While the natural exposure protocol involves a longer trial period than experimental challenge protocol, it has a lower workload and potentially a lower incidence of new IMIs. It also has a higher potential to be used within commercial farms as infections are naturally occurring.

Teat swabbing protocol

Swabbing teats before and after teat disinfection may be useful to determine the efficacy of teat disinfectant products. Studies by Gibson *et al.* (2008), Gleeson *et al.* (2009), Mišeikienė *et al.* (2015) and Baumberger *et al.* (2016) used a teat swabbing protocol to determine the efficacy of a range of premilking teat disinfectants. Swab samples of teats were taken using swabs moistened using buffered peptone water (Gibson *et al.* 2008; Baumberger *et al.* 2016). The actual sample can be collected in different ways such as wiping one side of the teat barrel from top to bottom, passing over the teat end and wiping the other side of the teat barrel from top to bottom (Baumberger *et al.* 2016), rubbing across the teat orifice and down the side of each teat avoiding contact with the udder hair or cows flank (Gleeson *et al.* 2009) or performed by triple rotary motions around the surface of the teat close to the tip from the front teat only (Mišeikienė *et al.* 2015). Different types of media have been used to store the sample swabs: 4 mL of buffered peptone water (Baumberger *et al.* 2016), 5 mL of sterile recovery medium 1.0 g/L protease peptone, 8.5 g/L sodium chloride and 2.0 g/L sodium thiosulphate (Gibson *et al.* 2008) or 5 mL of sterile tryptic soy broth (TSB) (Gleeson *et al.* 2009) with samples being kept at low temperatures (4 °C) (Gibson *et al.* 2008; Mišeikienė *et al.* 2015; Baumberger *et al.* 2016) or frozen (Gleeson *et al.* 2009; Baumberger *et al.* 2016; Separate to the reference (Gleeson et al. 2009; Baumberger et al. 2016) depending on time allowed between sampling and culturing of samples. The samples can be vortexed or agitated for 5–10 s (Gibson *et al.* 2008; Mišeikienė *et al.* 2015) or left unmixed (Gleeson *et al.* 2009; Baumberger *et al.* 2016). Swab samples are usually diluted to 10^{-2} to 10^{-3} for postdisinfectant swab samples (Mišeikienė *et al.* 2015; Baumberger *et al.* 2016) and 10^{-4} for predisinfectant swab samples (Baumberger *et al.* 2016).

Evaluation of disinfectant products using 'teat swabbing protocol'

In four reviewed studies, teat swabbing was used to compare the difference in bacterial counts on cows' teat skin after the implementation of a premilking hygiene regime. A study by Gleeson *et al.* (2009) used the teat swabbing protocol to evaluate a wide range of premilking teat disinfectants. In this study, disinfectant treatments showed different efficacies depending on whether cows were housed indoors or outdoors on pastures. Chlorine, iodine and wipes had a higher reduction of staphylococcal counts while cows were outdoors, with iodine also achieving a high reduction of streptococcal counts when used on cows indoors. Furthermore, Gibson *et al.* (2008) and Baumberger *et al.* (2016) identified a significant treatment by farm interaction for the reduction in all bacterial counts. This may be due to factors such as herd size, milk yield, field conditions, management techniques and housing (Gibson *et al.* 2008). Mišeikienė *et al.* (2015) used the teat swabbing protocol to evaluate a 0.2% w/w iodine disinfectant, 0.5% w/w aminopropyl-laurylamine disinfectant and a lactic acid-based disinfectant. The authors concluded that lactic acid and iodine had the highest probability of reducing total bacterial contamination, compared to 0.5% w/w aminopropyl-laurylamine. Baumberger *et al.* (2016) used the teat swabbing protocol to compare a 0.5% w/w iodine premilking disinfectant to a teat scrubber system which used chlorine dioxide. They found no differences in reduction between the treatments for most bacterial counts on teat skin.

Summary

The teat swabbing protocol can determine the efficacy of different premilking disinfectant treatments, by measuring the bacterial counts on the teat skin surface before and after treatment. A limitation of this method would be the high degree of variability in the numbers of recovered bacteria depending on the pressure applied to the teat from the swab, the choice of area on the teat, as the whole teat cannot be practically swabbed, and the variability in the surface area of the teat swabbed (Bade *et al.* 2008). In comparison with the natural exposure and experimental challenge protocols, the protocol does not give information on the reduction in new IMIs.

IN VITRO TESTING

There are a variety of laboratory test methods available to evaluate the efficacy or the antimicrobial activity of teat disinfectants against mastitis-causing bacteria. These methods include the excised teat protocol and agar diffusion assay (Balouiri *et al.* 2015).

Excised teat protocol

The excised teat protocol was used as a screening test to assess the teat disinfectants ability to reduce viable bacteria on teat skin surfaces (Nickerson *et al.* 2004), but in 1989, the membership of NMC decided to omit the excised teat model (Hogan et al. 1990). It was considered less time-consuming and less expensive than the other protocols described but was mainly used to determine whether an experimental product had the potential to be further evaluated as a teat disinfectant for cows. It involved using

excised teats from cows at slaughter, sorting and preparing teats for analysis by cleaning. Once clean, mastitis-causing pathogens are applied to the teat, followed by the test teat disinfectant (Nickerson 2001).

Excised teat preparation

To be accurate and reproducible, teat skin condition must be recorded and the teat prepared by trimming excess skin, washing in warm detergent, rinsed in sterile water, dried (Philpot *et al.* 1978; Schmidt *et al.* 1984; Boddie *et al.* 2002; Enger *et al.* 2015) and disinfected in 70% w/v ethyl alcohol (Boddie *et al.* 2002), 70% w/v isopropyl alcohol (Enger *et al.* 2015) or 70% w/v alcohol (type not stated) (Philpot *et al.* 1978; Schmidt *et al.* 1984). Teats can be placed in plastic bags in a glycerine and water solution and frozen at −20 °C until further use (Boddie *et al.* 2002; Enger *et al.* 2015). A range of 20–30 teats was used within the studies reviewed, with 10 teats used as negative control, 10 teats for the evaluation of the experimental product and, in some cases, 10 positive control teats. Teats were prepared by thawing firstly in warm water, dipping them in 70% w/v ethyl alcohol, dried with a paper towel and suspended by metal clips from a glass rod (Boddie *et al.* 2002).

Challenging teats and sample collection

Within four reviewed studies (Philpot *et al.* 1978; Schmidt *et al.* 1984; Boddie *et al.* 2002; Enger *et al.* 2015), test teats were dipped to a depth of 15 mm in the bacterial challenge, allowed 5 min to dry and then disinfected to a depth of 30 mm in the test product and left to dry for 10 min. Nondisinfected control teats were left to dry for 15 min. The preparation of the challenge suspension is very similar to challenge preparations outlined for the experimental challenge protocol. Within an excised teat protocol, a quench solution is used to recover bacteria from the teats to allow for culturing. Letheen broth is used within a quench solution to inactivate the disinfectant residue on the teat surface. Philpot *et al.* (1978) used sets of teats for a series of successive trials on the same day. Between trials, teats were rinsed in warm water for 2 min; dried and rinsed in 0.05% w/v sodium thiosulphate solution for 1 min; dried and rinsed in a 0.05% w/v lecithin and 0.05% w/v Tween 80 solution for 1 min; dried and rinsed in warm water for 1 min; dried and dipped in 70% w/v alcohol; and dried and resuspended. This was done to prevent any bacteria from the previous trial affecting results of successive trials. They concluded that the number of organisms recovered from teats in successive trials on the same day decreased. These reductions were not sufficient to effect log reductions but suggested that negative controls should be included for each successive trial.

When culturing the collected rinse samples, all reviewed studies diluted negative control samples to 10^{-4} and plated the treated teats undiluted when culturing samples, with Enger *et al.* (2015) also plating a dilution factor of 10^{-3} for negative controls.

Evaluation of disinfectant products using the 'excised teat protocol'

All studies reviewed evaluated a large range of products within their individual studies. This is beneficial as it allows comparison between products and aids in the selection of effective biocidal products. Watts *et al.* (1984) evaluated 38 different disinfectant products, which included 27 iodophors, one chlorhexidine and two quaternary ammonium compounds (QAC). Twenty-six of twenty-seven iodophor products achieved reductions >log 3 for *Staph. aureus*. Thirteen of the twenty-seven products, which contained 1% w/w iodophor, reduced *Str. agalactiae* by log 3 or greater. The QAC products yielded reductions >log 4 against *Staph. aureus* and *Str. agalactiae*. A 0.5% w/w cetylpyridinium chloride product had a log reduction of 4.64 and 4.71 for *Staph. aureus* and *Str. agalactiae*, respectively, and 1% w/w benzyl alcohol had a log reduction of 3.83 and 4.3 for *Staph. aureus* and *Str. agalactiae*, respectively.

Similarly, Philpot *et al.* (1978) carried out an evaluation of 13 teat disinfectants. Results show that the iodophors, sodium hypochlorite and sodium dichloro-s-triazinetrione were effective against a number of strains (*Staph. aureus*, *Str. agalactiae*, *E. coli* and *Pseudomonas aeruginosa*), with iodophor and chlorine products yielding the highest reductions. In comparison, QAC, chlorhexidine and cetylpyridinium chloride products were only found to be effective against *Staph. aureus* and *Str. agalactiae* and ineffective against *E. coli* and *P. aeruginosa*. Sodium hypochlorite was the most effective of all products. Sodium dichloro-s-triazenetrione was as effective as other disinfectants achieving log reductions between 4.92 and 5.31 for strains used. Similarly to Philpot *et al.* (1978) and Watts *et al.* (1984), Enger *et al.* (2015) looked at four different disinfectants (disinfectant A: 1% w/w H_2O_2, disinfectant B: 1% w/w chlorine dioxide, disinfectant C: 1% w/w iodophor and disinfectant D: 0.5% w/w iodophor) against *Staph. aureus*, *Str. agalactiae, Mycoplasma bovis, E. coli*, *Streptococcus dysgalactiae*, *Str. uberis*, *Staphylocccus xylosus*, *Staphylococcus epidermidis*, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus* and *Staphylococcus hyicus*. All four disinfectants yielded log reductions between 0.23 and 2.26.

Rather than evaluating the efficacy of a teat disinfectant against *Staph. aureus* and *Str. agalactiae*, Boddie *et al.* (2002) used six different products: (0.5% w/w iodine teat disinfectant, 0.5% w/w chlorhexidine teat disinfectant, two different chlorine teat disinfectants, a 0.5% w/w H_2O_2 mixed with 1.7% w/w lactic acid peroxygen teat disinfectant and QAC teat disinfectant) against *Mycoplasma* species. In this study, the average log reduction for all products was 6.29 for *Mycoplasma bovigenitalium*, 5.41 for *M. bovis* and 5.70 for *Mycoplasma californicum*. This result differed to

Enger *et al.* (2015), who found no significant difference between disinfectants used against *M. bovis*. A study by Boddie *et al.* (2002) found an average log reduction of 5.41 for *M. bovis* when products containing iodine, chlorhexidine, chlorine, 0.5% w/w H_2O_2 mixed with 1.7% w/w lactic acid peroxygen and quaternary ammonium were used. Unlike other studies, Schmidt *et al.* (1984) evaluated a sodium chlorite and lactic acid disinfectant diluted in water. The disinfectant yielded a high per cent log reduction for 14 of 21 strains tested (which included *Str. agalactiae*, *E. coli*, *Str. dysgalactiae*, *Str. uberis*, *Klebsiella oxytoca*, *Klebsiella pneumonia* and *P. aeruginosa)*. The test disinfectant was least effective against strains of *Staph. aureus*, as was the positive control (1% w/w iodophor).

Summary

From reviewing methods, the excised teat protocol is limited to showing the efficacy of the disinfectant and is used as a screening step for new products. The excised teat protocol could potentially give a better indication of efficacy when compared to other laboratory methods as they lack the characteristics of bovine teat skin (Philpot *et al.* 1978). The use of negative control teats allows for a comparison with the number of bacteria removed from the treated teats. A potential limitation of excised teat protocol is that it only measures the germicidal activity of teat disinfectants on the skin. The assay is incapable of measuring other important factors of a teat disinfectant such as the promotion of healing lesions, preventing chapping, persistent activity on the skin and formation of a barrier over the teat opening separate to the reference (orifice) (Schmidt et al. 1984). Also, evaluating the effectiveness against new IMIs caused by contagious, environmental and coliform bacteria is less predictable as the teats are not exposed to these organisms, not only during milking but between milkings (Schmidt *et al.* 1984). Furthermore, on comparing the studies by Schmidt *et al.* (1984) and Enger *et al.* (2015), both used the excised protocol as previously described by Philpot *et al.* (1978). Thus, this protocol has changed very little over the last 30 years which suggests that the protocol is a very effective measure of efficacy; however, few studies have used this method over the years.

Agar diffusion assay

The agar disc and agar well diffusion assays, also known as the Kirby Bauer method, have been mainly used for the detection of antibiotic-resistant bacteria (Biemer 1973). The assay has the potential to be used when determining the efficacy of teat disinfectants against different types of mastitis-causing bacteria and should be considered. Agar disc diffusion was developed in 1940 and is regarded as the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing (Biemer 1973; Balouiri *et al.* 2015). Agar plates are inoculated with a standardised inoculum of the test microorganism. Then, filter paper discs, which contain the disinfectant being tested, are placed on the surface of the agar (Biemer 1973). The Petri dishes are then incubated under suitable conditions. The disinfectant diffuses into the agar and inhibits the growth of the test microorganism. The diameters of the zones of inhibition are then measured, usually in mm (Biemer 1973; Balouiri *et al.* 2015). In both agar disc and agar well diffusion assays, a control disinfectant should be used that has a known zone of inhibition towards the test bacteria (Biemer 1973). Agar well diffusion follows the same principles as disc diffusion but instead of using discs containing the test disinfectant, wells of a certain diameter (6–8 mm) are punched into the agar and 10–200 μL of the test substance is put into each well. The experimental disinfectants diffuse into the agar, and inhibition zones are created, just like with the disc diffusion method. The diameter of the well and the zone of inhibition must all be measured to ensure accurate results. Factors such as rate of diffusion must also be taken into account (Balouiri *et al.* 2015).

Summary

Even though this assay is not a recommended protocol by the NMC, the assay has a high potential to effectively evaluate teat disinfectant products. Results could potentially be obtained quicker with a lower workload than the excised teat protocol.

CONCLUSIONS

Different protocols can be used to evaluate teat disinfectant products, which allow a full evaluation of the product from creation in a laboratory to use on a farm. The protocol chosen depends on the scope requirement of the investigation.

The experimental challenge protocol allows for the evaluation of the teat disinfectant against high levels of known bacteria. However, an experimental challenge study is more expensive in terms of an increased number of infected quarters and requires an increased workload as challenge suspensions must be prepared and tested daily. The natural exposure protocol is also time-consuming as it should be conducted over a full lactation to obtain representative results, but it has the potential of giving a better indication of the efficacy of the disinfectant within a farm environment. The use of noninferiority studies within the natural exposure protocol eliminates the use of negative controls (nondisinfected teats), which raise animal welfare and ethical concerns. The use of a split herd vs a split udder protocol may be more beneficial due to the interdependence of intramammary quarters. This could also help to reduce the impact on herd udder health and treatment methods. The teat swabbing protocol is limited due to a high degree of variability of bacteria recovered from teats and it also does not give an indication of the teat disinfectant or regimes'

potential to reduce new cases of IMIs. The excised teat protocol highlights the effectiveness of a disinfectant and indicates if the teat disinfectant should be further tested in an environment similar to where it will be used (i.e. natural exposure study). It is considered a reliable method of screening and evaluating teat disinfectants and is recommended by the NMC. However, the excised teat protocol has become less relevant in recent years, due to possible difficulties of acquiring, handling and disposing of teats. Agar diffusion has been used considerably less when evaluating teat disinfectants. However, agar diffusion is a less costly and time-consuming process as 3–4 teat disinfectants could be tested for each bacterial strain of interest on one agar plate. The inclusion of agar diffusion tests may allow manufacturers to identify efficient formulations at a faster rate.

Although not fully addressed within the review, the effect of each disinfectant product on teat condition and possible residues in the milk from that product must be considered when evaluating any product. Teat condition is mentioned within the NMC recommended protocols but the possibility of residues has not been addressed. Both of these topics require individual reviews of their own to fully ensure that products leave no harmful residues in milk and maintain good teat condition.

REFERENCES

Ats (2014) European Standards (EN) Teats Methods. [Internet document] URL http://ats-labs.com/testing-services/antimicrobial-test-library/european-standards-en-test-methods.

Bade R D, Reinemann D J and Thompson P D (2008) Method of assessing teat and udder hygiene. In: *2008 ASABE Annual international meeting*. Rhode Island.

Balouiri M, Sadiki M and Ibnsouda S K (2015) Methods for *in vitro* evaluating antimicrobial activity: a review. *Journal of Pharmaceutical Analysis* **6** 71–79.

Baumberger C, Guarin J F and Ruegg P L (2016) Effect of 2 different premilking teat sanitation routines on reduction of bacterial counts on teat skin of cows on commercial dairy farms. *Journal of Dairy Science* **99** 2915–2929.

Biemer J J (1973) Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. *Annals of Clinical and Laboratory Science* **3** 135–140.

Blowey R W and Edmondson P (2010) *Mastitis in Dairy Herds*, 2nd Ed, p 116–130. Wallingford, UK: CABI International.

Boddie R L, Owens W E, Ray C H, Nickerson S C and Boddie N T (2002) Germicidal activities of representatives of five different teat dip classes against three bovine *mycoplasma* species using a modified excised teat model. *Journal of Dairy Science* **85** 1909–1912.

Boddie R L, Owens W E, Foret C J and Janowicz P (2004) Efficacy of a 0.1% Iodine teat dip against *Staphylococcus aureus* and *Streptococcus agalactiae* during experimental challenge. *Journal of Dairy Science* **87** 3089–3091.

Bradley A J (2002) Bovine mastitis: an evolving disease. *The Veterinary Journal* **164** 116–128.

Ceballos-Marquez A, Hemling T, Rauch B J, Lopez-Benavides M and Schukken Y H (2013) Noninferiority trial on the efficacy of premilking teat disinfectant against naturally occurring new intramammary infections using a novel 2-step diagnostic process. *Journal of Dairy Science* **96** 8081–8092.

CellCheck (2012) *Mastitis Management Notes*. Animal Health Ireland.

Enger B D, Fox L K, Gay J M and Johnson K A (2015) Reduction of teat skin mastitis pathogen loads: differences between strains, dips and contact times. *Journal of Dairy Science* **98** 1354–1361.

Enger B D, White R R, Nickerson S C and Fox L K (2016) Identification of factors influencing teat dip efficacy trial results by meta-analysis. *Journal of Dairy Science* **99** 9900–9911.

Foret C J, Owens W E, Boddie R L and Janowicz P (2003) Efficacy of two iodine teat dips during experimental challenge with *Staphylococcus aureus* and *Streptococcus agalactiae*. *Journal of Dairy Science* **86** 3783–3786.

Foret C J, Corbellini C, Young S and Janowicz P (2005) Efficacy of two iodine teat dips based on reduction of naturally occurring new intramammary infections. *Journal of Dairy Science* **88** 426–432.

Gibson H, Sinclair L A, Brizuela C M, Worton H L and Protheroe R G (2008) Effectiveness of selected premilking teat-cleaning regimes in reducing teat microbial load on commercial dairy farms. *Letters in Applied Microbiology* **46** 295–300.

Gleeson D, O'Brien B, Flynn J, O'Callaghan E and Galli F (2009) Effect of pre-milking teat preparation procedures on the microbial count on teats prior to cluster application. *Irish Veterinary Journal* **62** 461–467.

Godden S M, Royster E, Knauer W, Sorg J, Lopez-Benavides M, Schukken Y, Leibowitz S and French E A (2016) Randomized noninferiority study evaluating the efficacy of a postmilking teat disinfectant for the prevention of naturally occurring intramammary infections. *Journal of Dairy Science* **99** 3675–3687.

Hogan J S, Galton D M, Harmon R J, Nickerson S C, Oliver S P and Pankey J W (1990) Protocols for evaluating efficacy of postmilking teat dips. *Journal of Dairy Science* **73** 2580–2585.

Lago A, Bruno D R, Lopez-Benavides M and Leibowitz S (2016) Short communication: efficacy of glycolic acid-based and iodine-based postmilking barrier teat disinfectants for prevention of new intramammary infections in dairy cattle. *Journal of Dairy Science* **99** 7467–7472.

Leslie K E, Petersson C S, Vernooy E and Bashiri A (2005) Efficacy of an iodophore teat disinfectant against *Staphylococcus aureus* and *Streptococcus agalactiae* in experimental challenge. *Journal of Dairy Science* **88** 406–410.

Leslie K E, Vernooy E, Bashiri A and Dingwell R T (2006) Efficacy of two hydrogen peroxide teat disinfectants against *Staphylococcus aureus* and *Streptococcus agalactiae*. *Journal of Dairy Science* **89** 3696–3701.

Mišeikienė R, Rudejevienė J and Gerulis G (2015) Effect of premilking antiseptic treatment on the bacterial contamination of cow teats' skin. *Bulgarian Journal of Veterinary Medicine* **18** 159–166.

Nickerson S C (2001) Choosing the best teat dip for mastitis control and milk quality. *NMC-PDPW Milk quality conference proceedings*, pp. 43–52. Madison, WI: National Mastitis Council, Inc..

Nickerson S C, Saxon A, Fox L K, Hemling T, Hogan J S, Morelli J, Oliver S P, Owens W E, Pawlak M and Petersson L (2004)

Recommended protocols for evaluating efficacy of postmilking teat germicides. *NMC Annual Meeting Proceedings* pp. 379–399. NC: Charlotte.

O'Brien B, Gleeson D and Jordan K (2013) Iodine concentrations in milk. *Irish Journal of Agricultural and Food Research* **52** 209–216.

Oliver S P, Lewis M J, King S H, Gillespie B E, Ingle T, Matthews K R, Dowlen H H, Drechsler P A, Wildman E E and Pankey J W (1991) Efficacy of a low concentration iodine postmilking teat disinfectant against contagious and environmental mastitis pathogens in 2 dairy herds. *Journal of Food Protection* **54** 737–742.

Oliver S P, Lewis M J, Ingle T L, Gillespie B E, Matthews K R and Dowlen H H (1993) Premilking teat disinfection for the prevention of environmental pathogen intramammary infections. *Journal of Food Protection* **56** 852–855.

Oura L Y, Fox L K, Warf C C and Kemp G K (2002) Efficacy of two acidified chlorite postmilking teat disinfectants with sodium dodecylbenzene sulfonic acid on prevention of contagious mastitis using an experimental challenge protocol. *Journal of Dairy Science* **85** 252–257.

Philpot W N, Boddie R L and Pankey J W (1978) Hygiene in the prevention of udder infections. IV. Evaluation of teat dips with excised cow teats. *Journal of Dairy Science* **61** 950–955.

Radostits O M, Fetrow J and Leslie K E (1994) *Herd Health: Food Animal Production Medicine*, 2nd Ed, p 397–435. Philadelphia, PA: W. B. Saunders.

Schmidt A L, Oliver S P and Fydenkevez M E (1984) Evaluation of experimental teat dip containing sodium chlorite and lactic acid by excised teat assay. *Journal of Dairy Science* **67** 3075–3080.

Schukken Y H, Rauch B J and Morelli J (2013) Defining standardized protocols for determining the efficacy of a postmilking teat disinfectant following experimental exposure of teats to mastitis pathogens. *Journal of Dairy Science* **96** 2694–2704.

Watts J L, Boddie R L, Pankey J W and Nickerson S C (1984) Evaluation of teat dips with excised teats. *Journal of Dairy Science* **67** 2062–2065.

Wesen D P and Schultz L H (1970) Effectiveness of a post-milking teat dip in preventing new udder infections. *Journal of Dairy Science* **53** 1391–1403.

