

## EVALUATION OF SOME DISINFECTANTS ON TOTAL MICROBIAL LOAD IN TEATS CUPS AND MILK

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### ABSTRACT

Investigation was carried out at the farm of Animal Production Department, College of Agriculture and Forestry, Mosul University on 20 Dairy Friesian Cows. The Cows were milked twice daily in their stands with an Alfa Laval pipeline milking machine. Teats Cups were treated by disinfectants as follows: 1-Untreated Teat Cups 2- Wet towel washing water (55-60C0) followed by paper towel drying. 3- Wet towel washing (55-60C0) then by 0.1:1000, 5:100, 1:100 Iodophor respectively, followed by paper towel drying. 4-- Wet towel washing (55-60C0) then by Hibitane at a concentration of 1%, 0.5%, 0.25%, respectively followed by paper towel drying. 5- Wet towel washing (55-60C0) then by half strength Lukewarm potassium at a concentration 0.5:10000, 1:10000 respectively followed by paper towel drying, All these treatments were applied onto teats cups for 30-60 seconds.

The aims of this study is to evaluate the effects of these disinfectants on Colony forming units (CFUs) of teats cups swab samples and Colony forming units (CFUs) of milk samples. The lowest percentage in the reduction of Log<sub>2</sub> /swab of teats cups surface was noticed when wet towel washing (55-60C0), followed by paper towel drying being 36.05%. By using Iodophor there was an increase of about 20% in the reduction of TBC/swab (Total Bacterial Count) with each increase in Iodophor concentration, and were 58.5%, 79.3% and 95.7% with concentrations of 1:100, 0.5:100 and 0.1:100). The highest concentration of Hibitane gave a reduction in TBC/swab similar to that of the highest Iodophor concentration, being 94.5%. A decline in the percentage of TBC/swab was proportional with the decrease in Hibitane concentration, being 62.6% and 42.1%, when Hibitane was used at concentrations of 0.5% and 0.25% respectively. Potassium permanganate treatments show a slightly better than wet towel treatments, being 44.9% and 46.9% in concentrations 0.5:10000 and 1:10000 respectively.

The reduction percentage of Log<sub>2</sub> TBC/ ml of milk in different treatments was noticed when wet towel washing (55-60C0), followed by paper towel drying being 30.0%. By using Iodophor there was an increase of about 20% in the reduction of TBC/swab with each increase in Iodophor concentration, and were 56.25%, 79.0% and 95.5% with concentrations of 1:100, 0.5:100 and 0.1:100). Hibitane concentrations gave a reduction in TBC/swab lower than that given by Iodophor, being 75.4%. A decline in the percentage of TBC/swab was proportional with the decrease in Hibitane concentration, being 47.3% and 39.5%, when Hibitane was used at concentrations of 0.5% and 0.25% respectively. Potassium permanganate treatments show a slightly better than wet towel treatments, being 40.5% and 49.7% in concentrations of 0.5:10000 and 1:10000 respectively.

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## INTRODUCTION

Premilking cow preparation is proven to be an important step in achieving maximum milk yield, quality and udder health (Jeffrey and John .,1995) .The key to success for the production of good quality milk and safe for human consumption requires pay attention to the health of the udder and teats. As the udder is the factory which manufactures the milk and that the cleanliness of the udder and teats and teats cups have a great role in the production of high quality milk free of germs( Stephen .,2009). The progress made in raising the rates of public health makes it imperative for dairy producers to increased attention to the quality of their products, because consumers can distinguish between good and poor quality of the milk. As is known, good milk production and the subsequent manufacturing steps starting from the farm (Trevor and Richard.,2008).The milk which produced from clean and healthy animals and under sanitary conditions usually contains a few number of bacteria (Jeffrey and John .,1995). Bacteria in milk may gain entrance from the surrounding environment, udder flora and from intramammary infections (Murphy and Boor., 2000). Because the udder and teats are the surfaces which contact with the ground and exposed to the atmosphere, which can contaminate milk, dung, dirt and bacteria, so the washing and cleaning and disinfecting the outer surface of the udder and teats is necessary to prevent contamination of milk (Knappstein.,2004) .Milk quality largely depends on exclusion of contaminant bacteria and extraneous chemicals. Cleaning milking machines, udder and teats between milking is of vital importance in reducing udder infections. Since, mastitis causing microorganisms like *Streptococcus agalactiae* are very important in significantly increasing total bacterial count affecting milk quality which may persist even after removal of causative agent. Udder and teat surface bacterial contaminants especially *Staphylococcus* species, are the third source of milk bacterial contaminants. Moreover, chemicals contaminating milk is a source for residue that should be monitored for accreting good milk quality. Nowadays premilking hygiene highly stressed on the significant decreasing in the number of mastitic bacteria on the teat end These include udder washing with water hose, with or without sanitizer; wet towel washing of teats, with or without sanitizer; and each of these methods with or without paper towel drying. Drying udder and teats after washing with water or sanitizers, give the lowest standered plate counts, coli forms and staphylococcus spp. In the food industry, as in medical practice, preparations containing germicides are used on the hands of personnel to reduce the carriage of potential pathogens (Hall .,1969). Moist heat (water at below or above 100Co could be used for disinfection processes like water at boiling temperature, or less than boiling ,i.e., at pasteurization (Sridar.,2008).Iodophor, Hibitane and potassium permanganate are all used in udder and teats cups washing. Iodine solutions or tinctures have been used by health professionals primarily as antiseptics on skin or tissue. Iodophor , on the other hand, have been used both as antiseptics and disinfectants. An Iodophor is a combination of iodine and a solubilizing agent or carrier; the resulting complex provides a sustained-release reservoir of iodine and releases small amounts of free iodine in aqueous solution. This product and other Iodophors retain the germicidal efficacy of iodine but unlike iodine generally are non-staining and relatively free of toxicity and irritancy (Gottardi.,1991,2001). Chlorhexidine is an antiseptic effective

against a wide variety of gram-negative and gram-positive organisms, facultative anaerobes, aerobes, and yeast. Chlorhexidine is used as an ingredient of bacteriostatic and bactericidal by the activity of membrane disruption in a generalpurpose skin general skin cleansers, surgical scrub, germicidal hand rinses and animal disinfection products. (Block.,2001). Chlorhexidine is an antiseptic with antibacterial, antifungal and some antiviral activity; used in skin cleansers for surgical scrub, preoperative skin preparation, cleansing skin wounds and teat dips. Used as the acetate, gluconate or hydrochloride salts. Proprietary names are Hibitane, (Mosby., 2004).

### MATERIALS AND METHODS

The experimental was carried out at the farm of Animal Production Department, College of Agriculture and Forestry, Mosul University on 20 Dairy Cows. The Cows were milked twice daily in their stands with an Alfa Laval pipeline milking machine. The experiment lasted from the period December 2007 to January 2008.

Udder treatments:

Teat Cups were treated by disinfectants as follows:1-Untreated Teat Cups 2- Wet towel washing water (55-60C0) followed by paper towel drying. 3- Wet towel washing (55-60C0) then by 0.1:1000, 5:100,1:100 Iodophor respectively , followed by paper towel drying. 4-- Wet towel washing (55-60C0) then by Hibitane at a concentration of 1% , 0. 5% , 0.25%, respectively followed by paper towel drying. 5- Wet towel washing (55-60C0) then by half strength Lukewarm potassium at a concentration 0.5:10000 , 1:10000 respectively followed by paper towel drying, All these treatments were applied onto teats cups for 30-60 seconds.

Sampling:

Twenty swab and twenty milk samples of treated and non-treated teats cups were collected from cows reared at the college of Agriculture and ,during the period December 2007 to January 2008, according to (Zain and Bradbury 1995) as follows:

Sterile cotton swabs immersed in peptone saline solution were used for sampling. Swabs were streaked on five locations of an area of 16 cm<sup>2</sup> and placed in test tubes containing peptone saline, and transferred to the veterinary public health laboratory (College of veterinary medicine) in a cool box within hour for enumeration of TBC/swab or ml of milk on nutrient agar.

Decimal serial dilution 10<sup>-1</sup>-10<sup>-6</sup> were prepared, and the method of(John.,1997) was followed for counting TBC/swab or milk according to the formula:

$$\text{TBC/swab or 1 ml of milk} = \frac{\text{No. Of calculated colonies} \times 1 / \text{Dilution}}{\text{Volume of diluents 'for spreading}}$$

### RESULTS

Total Bacterial Count( TBC) of teat cups swab samples:

The Log<sub>2</sub> of TBC/ swab of untreated teats cups was significantly ( $p \leq 0.05$ ) differ from those swab samples when teat cups were treated with wet towel washing (55-60C0), followed by paper towel drying , and in the same time there was significant difference between the Log<sub>2</sub> of TBC/ swab before and after teats cups treatment with wet towel washing (55-60C0), followed by paper towel drying(Table 1).

Table (1) Log<sub>2</sub> of TBC/swab of teats cups before and after water treatments

Treatment	Log <sub>2</sub> TBC/swab before udder treatment	Log <sub>2</sub> TBC/swab after udder treatment
T1	5.518 a	A 5.518 a
T2	5.633 a	B 3.602 b

By using Iodophor, the results show that there was in general significant ( $p \leq 0.05$ ) reduction in the Log<sub>2</sub> of TBC/ swab before and after teat cups treatment with all used concentrations of Iodophor, and there was also significant ( $p \leq 0.05$ ) reduction in the Log<sub>2</sub> of TBC/ swab between different Iodophor concentrations, with a proportional reduction in the Log<sub>2</sub> TBC/ swab with each increase in the Iodophor concentration (Table 2).

Table (2) Log<sub>2</sub> of TBC/swab of teats cups before and after Iodophor treatments

Treatment	Log <sub>2</sub> TBC/swab before udder treatment	Log <sub>2</sub> TBC/swab after udder treatment
T3	5.531 a	A 2.301 b
T4	5.556 a	B 1.150 b
T5	5.447 a	C 0.231 b

Treatments with Hibitane and in all of its concentrations, it was shown that there was a significant ( $p \leq 0.05$ ) reduction in the Log<sub>2</sub> of TBC/ swab before and after teats cups udder treatments, and there were also proportional and significant ( $p \leq 0.05$ ) reduction in Log<sub>2</sub> TBC/ swab with each increase in Hibitane concentration (Table 3).

Table 3 Log<sub>2</sub> of TBC/swab of teats cups before and after Hibitane treatments

Treatment	Log <sub>2</sub> TBC/swab before udder treatment	Log <sub>2</sub> TBC/swab after udder treatment
T6	5.505 a	C 0.298 b
T7	5.440 a	B 2.034 b
T8	5.491 a	A 3.176 b

Potassium permanganate have also as other above treatments a significant ( $p \leq 0.05$ ) reduction effect in the Log<sub>2</sub> of TBC/ swab before and after teats cups treatments, and also proportional and significant ( $p \leq 0.05$ ) reduction in Log<sub>2</sub> TBC/ swab of the udder with each increase in potassium permanganate concentrations (Table 4).

Table (4) Log<sub>2</sub> of TBC/swab of teats cups before and after potassium permanganate treatments.

Treatment	Log <sub>2</sub> TBC/swab before udder treatment	Log <sub>2</sub> TBC/swab after udder treatment
T9	5.716 a	A 3.146 b
T10	5.568 a	B 2.954 b

Total Bactrial Count (TBC) of milk samples:

The Log<sub>2</sub> of TBC/ ml of milk of untreated udders was significantly ( $p \leq 0.05$ ) differ from those milk samples when udder was treated with wet towel washing (55-60C0), followed by paper towel drying, and in the same time there was significant difference between the Log<sub>2</sub> of TBC/ ml of milk before and after udder treatment with wet towel washing (55-60C0), followed by paper towel drying (Table 5).

Table (5) Log<sub>2</sub> of TBC/ml of milk before and after water treatments

Treatment	Log <sub>2</sub> TBC/ml of milk before udder treatment	Log <sub>2</sub> TBC/ ml of milk after udder treatment
T1	3.342 a	A 3.342 a
T2	3.819 a	B 2.672 b

By using Iodophor , the results show that there was in general significant ( $p \leq 0.05$ ) reduction in the Log<sub>2</sub> of CFU/ ml of milk before and after udder treatment with all used concentrations of Iodophor , and there was also significant ( $p \leq 0.05$ ) reduction in the Log<sub>2</sub> of TBC/ ml of milk between different Iodophor concentrations, with a proportional reduction in the Log<sub>2</sub> TBC/ ml of milk with each increase in the Iodophor concentration( Table 6).

Table (6) Log<sub>2</sub> of TBC/ml of milk before and after Iodophor treatments.

Treatment	Log <sub>2</sub> TBC/ml of milk before udder treatment	Log <sub>2</sub> TBC/ ml of milk after udder treatment
T3	3.662 a	AB 1.602 b
T4	3.826 a	B 0.801 b
T5	3.556 a	A 0.160 b

Treatments with Hibitane and in all of its concentrations, it was shown that there was a significant ( $p \leq 0.05$ ) reduction in the Log<sub>2</sub> of TBC/ ml of milk before and after udder treatments, and there were also significant ( $p \leq 0.05$ ) reduction in Log<sub>2</sub> TBC/ ml of milk with the highest Hibitane concentration (3%), compared with the other lower two concentrations (0.75% and 0.37%) (Table 7).

Table (7) Log<sub>2</sub> of CFU/ml of milk before and after Hibitane treatments.

Treatment	Log <sub>2</sub> TBC/ml of milk before udder treatment	Log <sub>2</sub> TBC/ ml of milk after udder treatment
T6	3.785 a	B 2.00 b
T7	3.653 a	B 2.079 b
T8	3.838 a	A 3.380 b

Potassium permanganate treatments with its two concentrations showed no differences in Log<sub>2</sub> /ml of milk before and after udder treatments or between its two concentrations (Table 8).

Treatment	Log <sub>2</sub> TBC/ml of milk before udder treatment	Log <sub>2</sub> TBC/ ml of milk after udder treatment
T9	3.994 a	A 3.591 ab
T10	3.997 a	A 3.554 ab

The lowest percentage in the reduction of Log<sub>2</sub> /swab was noticed when wet towel washing (55-60C0), followed by paper towel drying being 36.05%. By using Iodophor there was an increase of about 20% in the reduction of TBC/swab with each increase in Iodophor concentration, and were 58.5%,79.3% and 95.7% with concentrations of 1:100, 0.5:100 and 0.1:100). The highest concentration of Hibitane gave a reduction in TBC/swab similar to that of the highest Iodophor concentration, being 94.5%.Adecline in the percentage of TBC/swab was proportional with the decrease in Hibitane concentration, being 62.6% and 42.1% , when Hibitane was used at concentrations of 0.5% and 0.25% respectively. Potassium permanganate treatments show a slightly better than wet towel treatments, being 44.9% and 46.9% in concentrations 0.5:10000 and 1:10000 respectively.

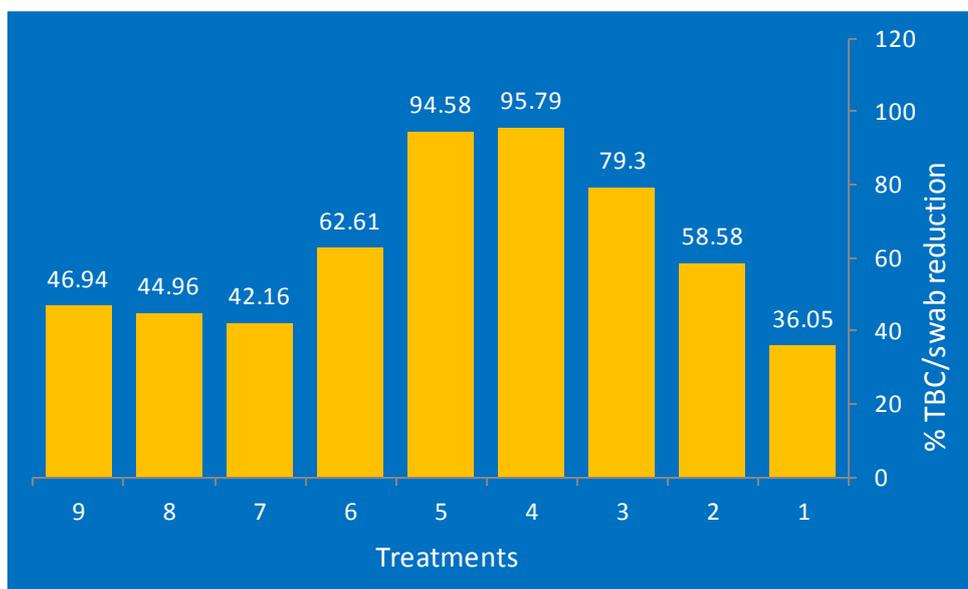


Figure (1) Percentage reduction of Log<sub>2</sub> TBC/ swab of teats cups in different treatments.

Reduction percentage of LOG<sub>2</sub> TBC/ ml of milk in different treatments:

The lowest percentage in the reduction of Log<sub>2</sub> /swab was noticed when wet towel washing (55-60C0), followed by paper towel drying being 30.0%. By using Iodophor there was an increase of about 20% in the reduction of TBC/swab with each increase in Iodophor concentration, and were 56.25%,79.0% and 95.5% with concentrations of 1:100, 0.5:100 and 0.1:100). Hibitane concentrations gave a reduction in TBC/swab lower than that given by Iodophor , being 75.4% . Adecline in the percentage of TBC/swab was proportional with the decrease in Hibitane concentration, being 47.3% and 39.5% , when Hibitane was used at concentrations of 0.5% and 0.25% respectively. Potassium permanganate treatments show a slightly better than wet towel treatments, being 40.5% and 49.7% in concentrations of 05.10000 and 1:10000 respectively.

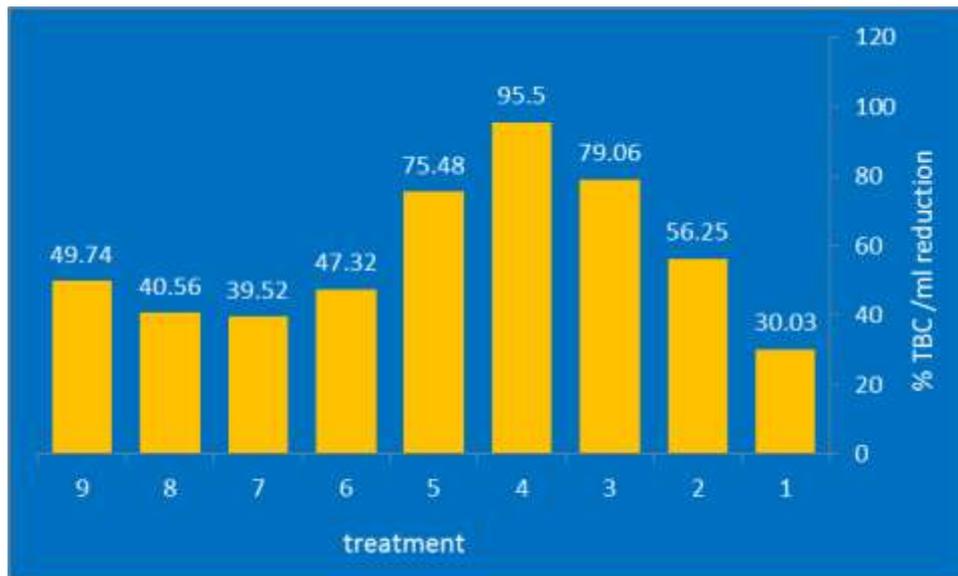


Figure (2) Percentage reduction of Log<sub>2</sub> TBC/ ml of milk in different treatments.

## DISCUSSION

According to the field of application, strategies for the prevention of the transfer of microbial skin flora from the udders and teat cups must consider the various categories of flora: transient, resident or stemming from infected lesions on the udder (infection flora). Depending on the species and virulence of the microorganism and of the susceptibility of the infection target, transient flora may or may not be of pathogenic importance. In contrast, resident skin flora is usually regarded as pathogenic. Microorganisms stemming from infected lesions are of proven pathogenicity. Only the transient and infection flora from the udders play a role. Milk may be rendered safe by procedures for the elimination of transients such as hygienic udder wash by hand rub (in the order of increasing efficacy). Furthermore, the duration of treatment (between 30 and 60 s) significantly influences the achievable reduction of microbial release. According to the new European standards (CEN) for testing chemical disinfectants and antiseptics, products for hygienic udder wash must be significantly more efficacious than contaminated udders. There exists a strong positive correlation of the reduction of microbial release in milk and the duration of udder treatment. Some of them exert a bacteriostatic sustaining. This, however, is not necessary with the latter as the initial bacterial reduction is that strong that restitution of the udder skin flora takes > 3 hours (Rotter .,1996).

The results of this study showed that the treatment of teats cups with towel moistened in warm water at 55-60°C was able to reduce TBC/swab by two Log<sub>2</sub> only, which is the lowest reduction (36%) of teats cups contaminating flora among all other treatments. This temperature is used for disinfecting serum and vaccines from contaminating bacteria. Although there was a significant reduction in TBC/swab in treatment with water at this temperature, compared to pre-treatment TBC/swab, but it should be said that not all contaminating bacteria were completely eliminated through the action of coagulation and denaturation of bacterial proteins, since it should be worked for 1 hour to inactivate vegetative bacteria but not spores(

Srider .,2008). In this study we prepare more concentrated dilutions to compensate the shorter period of contact on teats cups, in order not to take more time waiting for milking. The best results in reduction of teats cups contaminating flora was achieved by applying Iodophor, compared to other chemicals used. There was 5,4 and 3 Log<sub>2</sub> reduction in TBC/swab of teats cups contaminating bacteria when Iodophor was used at 1:100, 0.5:100 and 0.1:100 concentrations when comparison is made between pre and post treated teats cups. The percentage of reduction was proportional with each increase in Iodophor concentration, from 58.5% to 95.7%. Published reports on the in vitro antimicrobial efficacy of Iodophors demonstrate that Iodophor are bactericidal, fungicidal and virucidal (Rutala et al.,1991). Three brands of povidone-iodine solution have demonstrated more rapid kill (seconds to minutes) of *S. aureus* and *M. chelonae* at a 1:100 dilution than did the stock solution( Berkelman et al .,1982). Iodine compounds have the widest spectrum of anti-infective against bacteria, fungi, spores, protozoa, viruses, and yeasts. Aqueous iodine are less effective than alcoholic solutions, but alcoholic component is drying and irritating to abraided skin. Iodophor is convenient to use as it is less irritating, but not as effective. In the second order was with Hibitane application, and as Chlorhexidine is used as a safe antiseptic or disinfectant to apply to prevent body infection in the form of acetate, gluconate or hydrochloride, so it is in this study it gave a reduction of 5, 3 and 2 Log<sub>2</sub> of TBC/swab of the contaminating teats cups flora, was noticed by using 1, 0.5 and 0.25% of Hibitane treatment of udder surface. The percentage of TBC /swab reduction were 94.5%, 62.6 % And 42.1% respectively. In clinics, skin germicides are used to reduces skin carriage of potential pathogenic bacteria like chlorhexidine {Hibitane) and Iodophor are used, and chlorhexidine in food premises appear to reduce skin bacteria and carriage of potential pathogens (Hall.,1969). Antimicrobial activity of 0.2%, 1%, and 2% chlorhexidine gluconate was tested against *Staphylococcus aureus* and *Candida albicans*, and found that 2.0% gel and liquid formulations eliminated *Staphylococcus aureus* and *Candida albicans* in 15 seconds, whereas the gel formulation killed *Enterococcus faecalis* in 1 minute (Morgana et al ., 2004). . By this, the achievable average reduction of the microbial release ranges between 2.0 and 2.4 log<sub>2</sub>. In contrast, antiseptic washing procedures with preparations containing low concentrations of Iodophor s, chlorhexidine gluconate reduce the bacterial release within 2-5 min only by 0.5 to 1.2 log<sub>2</sub> (Rotter .,1996).The efficacy of Iodophor germicides containing different concentrations of available iodine against natural teats cups microflora when compared with chlorhexidine gluconate (0.25 to 1%) liquid detergent (Hibitane ), non-germicidal hot water rinse. The tap water rinse was ineffective compared with all other treatments, because it reduce TBC/swab of contaminating bacteria only 2Log<sub>2</sub>, and do not reach 4 Log<sub>2</sub> reduction as Iodophor and Hibitane. Only 1% chlorhexidine gluconate liquid detergent and Iodophor at 1:100 concentration were significantly better than other concentration of both chemicals, These agents caused a significant reduction in the number of 'natural' microorganisms released from teat cups after a standard 30-60 s udder wash. The low-concentration Iodophor products and chlorhexidine gluconate failed to give satisfactory results of 4.2 to 4.4 Log<sub>2</sub> reduction in TBC/swab of natural teat cups contaminating bacteria, since they should be applied for more

longer period of contact, 2-3 minutes (Stiles and Sheena .,1985) . The germicidal effects of potassium permanganate and eosin were not satisfactory, since they were near to that of warm water. This could be due to short contact time of 30-60s instead of 2-3 minutes. The picture of TBC/ml of milk is a mirror to that of TBC/swab on the teat cups. A slightly similar picture was also obtained in the percentage of reduction in TBC/ml of milk. The standard aerobic plate TBC/swab in teats cups treatments and TBC/ml of milk was highly correlated ( $r = 0.01$ ). Wet towels treatment was effective in reducing TBC/ml of milk, with a percentage of 30.0 %. The higher concentration of Iodophor (1:1000), was the one which significantly reduce CFU/ml of milk (95.5%), among the other two concentration which were similar in their significance reduction of TBC/ml of milk (79.0 and 56.2 respectively). The same picture in TBC/swab reduction was noticed through using different concentrations of Hibitane to that of Iodophor, but the difference was in the rate of reduction which was higher in case of Iodophor than that recorded for Hibitane. No significant differences in the reduction of TBC/ml of milk or in the percentage of this reduction was revealed by using potassium permanganate in the hygienic treatment of udder before milking. In conclusion, it is clear that Iodophor is the most effective among the three chemicals used, although all of them can be used for udder washing before milking process. The application of these chemicals in hygienic treatment of udder surfaces is worth to be applied in the practical milking process.

#### تقييم تأثير بعض المعقمات على الحمل الجرثومي الكلي في كؤوس الحلمات وحليب الأبقار

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#### الخلاصة

اجريت هذه الدراسة في الحقل التابع لقسم الانتاج الحيواني / كلية الزراعة والغابات / جامعة الموصل على 20 بقرة فريزيان حيث كانت الأبقار تحلب مرتين باليوم باستخدام جهاز الحلب الأوتوماتيكي نوع الفا حيث عوملت كؤوس الحلمات والضرع بالمعقمات وكالاتي:  
كؤوس حلمات غير معاملة.  
الغسل بالماء درجة حرارته 55-60 درجة مئوية ومن ثم تم تجفيفه باستخدام ورق تجفيف.  
الغسل بالماء درجة حرارته 55-60 درجة مئوية ومن ثم المسح بمطهر الايودوفور ذات تراكيز مختلفة 0.1، 0.5، 1 مل/100 مل ومن ثم تم تجفيفها باستخدام ورق التجفيف.  
الغسل بالماء درجة حرارته 55-60 درجة مئوية ومن ثم المسح بمطهر الهيبتانين ذات تراكيز مختلفة 1، 0.5، 0.25 مل/100 مل ومن ثم تم تجفيفها باستخدام ورق التجفيف.  
الغسل بالماء درجة حرارته 55-60 درجة مئوية ومن ثم المسح بمطهر برممنكات البوتاسيوم ذات تراكيز مختلفة 0.5، 1 مل/10000 مل ومن ثم تم تجفيفها باستخدام ورق التجفيف.  
استغرقت كل معاملة من المعاملات المذكورة اعلاه على كؤوس الحلمات لمدة 30-60 ثانية. والهدف من الدراسة هو لتقييم تأثير هذه المعقمات المذكورة اعلاه على العدد الكلي للجراثيم الحية (وحدة مكونة للمستعمرات /مل) لكؤوس الحلمات وعينات الحليب.  
اظهرت النتائج ان اقل نسبة في خفض العدد الكلي للجراثيم الحية في كؤوس الحلمات كان عند الغسل بالماء درجة حرارته 55-60 درجة مئوية ومن ثم تم تجفيفه باستخدام ورق تجفيف 36.05 % وباستخدام الغسل بالماء درجة حرارته 55-60 درجة مئوية ومن ثم المسح بمطهر الايودوفور زادت نسبة الخفض العدد الكلي للجراثيم الحية في كؤوس الحلمات بارتفاع نسبة التركيز حيث كانت النتائج كالاتي 95.7%، 79.5%، 58.5% عند استخدام التراكيز 0.1، 0.1 مل/100 مل وعلى التوالي. ان اعلى تركيز للهيبتانين كان قد

اعطى انخفاضا في العدد الكلي للجراثيم الحية يعادل ما احده اعلى تركيز للايودوفور اذ بلغ 94.5 % وقد انخفض تأثير الهيبتاتين على العدد الكلي للجراثيم الحية مع انخفاض تركيزه حيث بلغ 42.1%، 92.6% عند استخدام الهيبتاتين بتركيز 0.25%، 0.5% واطهر برمنكنات البوتاسيوم نتائج افضل من المسح بالماء اذ انخفض العدد الكلي للجراثيم الحية بنسبة 46.9%، 44.9% عند التراكيز 0.5، 1 مل/10000 مل على التوالي. اما بما يخص نسبة الانخفاض في العدد الكلي للجراثيم الحية في عينات الحليب في حالة الغسل بالماء درجة حرارته 55-60 درجة مئوية ومن ثم تم تجفيفه باستخدام ورق تجفيف فقد بلغت 30% وباستخدام الايودوفور لوحظ انه هناك زيادة في انخفاض العدد الكلي للجراثيم الحية في عينات الحليب بزيادة تركيزه اذ بلغت 99.5%، 70%، 56.25% وبتراكيز 1، 0.5، 0.25 مل/100 مل على التوالي. نسبة انخفاض العدد الكلي للجراثيم الحية في عينات الحليب كان اقل عند استخدام الهيبتاتين مقابل الايودوفور حيث بلغت 75% عند استخدام اعلى تركيز وانخفضت النسبة الى 39.5%، 47.3% عند انخفاض تراكيز الهيبتاتين من 0.5%، 25.0% على التوالي كما اوضحت النتائج ايضا ان استخدام برمنكنات البوتاسيوم كانت افضل قليلا من الغسل بالماء درجة حرارته 55-60 درجة مئوية ومن ثم تم تجفيفه باستخدام ورق تجفيف اذ بلغت 40.5%، 49.7% عند التراكيز 0.5، 1 مل/10000 مل على التوالي.

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