

Evaluation of two chlorhexidine - alcohol based skin disinfectants in blood donation setting

B.K.L. So
C.C.Y. Chu
P.L. Ho*
K.H. Chow*
J.N.S. Leung
I.Y.M. Lee
C. K. Lin
C. K. Lee

Hong Kong Red Cross Blood Transfusion Service, *Carol Yu Center for Infection and Department of Microbiology, the University of Hong Kong

Correspondence: Dr. C.K. Lee, Hong Kong Red Cross Blood Transfusion Service, 15, King's Park Rise, Hong Kong, SAR, China.

Tel: +852-2710-1271

Fax: +852-2780-4151

Email: ckleea@ha.org.hk

Keywords: chlorhexidine, skin disinfection, bacterial contamination, risk, blood donation

Running title: Evaluation of chlorhexidine- alcohol based skin disinfectants

Declaration of Conflict of Interest

The authors declared no conflicts of interest in the study.

Abstract

Background:

Source reduction is important in minimizing bacterial contaminated risk of blood products but previous evaluation of chlorhexidine (CHX) was confounded by inability of **Tween** and lecithin to neutralize CHX. The study aims to address this limitation and also evaluate the effectiveness of two CHX–alcohol based skin disinfectants in blood donation setting.

Methods:

A two-stage observational study was conducted. A single step 2% chlorhexidine gluconate/70% isopropyl alcohol brush (CHX/IPA-1) was first compared with current skin disinfection procedure consisting of sequential application of 10% povidone iodine and 70% isopropyl alcohol (PI/IPA). Standard plates with conventional neutralizers (0.3% Tween 80, 0.1% lecithin) were used to enumerate residual bacterial counts. Then CHX/IPA-1 was compared with another applicator CHX/IPA-2 with identical disinfectant contents using in-house plates with neutralizers (3% Tween 80, 0.3% lecithin, 0.1% histidine, 0.5% sodium thiosulphate, 3% saponin, 1% ether sulphate) having enhanced ability to neutralize CHX.

Results:

All three products were found to reduce plate counts by $> 2 \log_{10}$ after disinfection.

The CHX/IPA-1 group gave fewer residual bacterial growth on standard plates than PI/IPA group (5.9% vs 61.7%, $P < 0.001$). With the use of in-house plates, residual bacterial growth were of no difference in both CHX/IPA-1 and CHX/IPA-2 groups (42.5% vs 49.4%, $P=0.26$).

Conclusion:

Good efficacy was observed with one stage application of CHX/IPA in pre-donation skin disinfection and it could replace PI/IPA. However, the efficacy of CHX/IPA could be grossly overestimated in testing with standard plates because of insufficient neutralization.

Background

Transfusion associated bacterial infection remains a major risk in clinical settings [1-5]. Over the years, transfusion communities have introduced various strategies in reducing the risk. These include improved skin disinfection [6-8], adoption of diversion pouch [8-11], implementation of pre-release bacterial surveillance tests [12-14] and pathogen reduction technologies [15-18]. Theoretically, some combination of them could have the maximal effectiveness but may add operational complexity and/or cost.

Source elimination should remain the most fundamental step in limiting bacteria entering into the system. While a good health history screening of prospective donors should always been in practice to reduce transfusion transmitted bacterial and viral infection, it is by no mean exhaustive and some donors may not realize a risk exposure history e.g. recent diarrhoea may result in asymptomatic bacteria carrier stage. For bacterial risk, skin disinfection and diversion pouch are two main approaches currently in practice. The latter is almost universally implemented in most developed countries. On the other hand, skin disinfection protocol varies from country to country.

Recently, 2% chlorhexidine gluconate/70% isopropyl alcohol formulation (CHX/IPA) emerged to be at least as effective as other disinfectants [19-25]. As commercial

available applicator appears to be simpler to handle, some blood centers, after the validation, have implemented it as route skin disinfection [21, 24]. Therefore, the study is designed to evaluate the residual bacterial load after skin disinfection by two commercial available CHX/IPA applicators.

Methods:

The study was conducted in two parts at the Hong Kong Red Cross Blood Transfusion Service when the currently used skin disinfectant i.e. 10% povidone-iodine/70% isopropyl alcohol (PI/IPA) was first compared with a commercially available 2% chlorhexidine gluconate/70% isopropyl alcohol (CHX/IPA) brush (named as CHX/IPA-1) in part one and then two preparations of CHX/IPA (CHX/IPA-1 brush and CHX/IPA-2 swab) were studied in part two.

Skin disinfectant (PI/IPA) consisted of 10% povidone-iodine swabstick (Professional Disposables International Inc, NY, US) and 70% isopropyl alcohol swab (Professional Disposables International Inc, NY, US). Both CHX/IPA-1 and CHX/IPA-2 contained 2% chlorhexidine gluconate/70% isopropyl alcohol. They were Chloraprep® 3 ml brush (Cardinal Health Inc, KS, US) and SOLU-I.V. swabstick (SOLUMED, 3M Canada Company, Ontario, Canada)

Subjects were recruited into the studies based on the sample size estimation below.

The study has been approved by Research Ethics Committee of Kowloon East and Central Clusters, Hospital Authority, Hong Kong.

In part one, one arm of each subject would be either disinfected by PI/IPA or CHX/IPA-1 whereas the arm of contralateral side served as comparison of the baseline bacterial count. Before disinfection, a baseline bacterial count was

enumerated by applying contact plate on both arms. Then, the selected arm was disinfected by PI/IPA or CHX/IPA-1 under a defined protocol (see below). Upon completion, the skin was air dried, another contact plate was applied to determine the residual bacterial count. In part two, both arms of each subject were disinfected by CHX/IPA-1 or CHX/IPA-2 with the same steps to enumerate bacterial counts before and after the procedure.

Skin disinfection procedure

For each arm, at least 4 x 4cm area of the antecubital fossa was chosen as intended “venepuncture site”. **The site was first sampled by culture plate with a contact time of 10 seconds (before disinfection) which allowed maximal contact between skin and agar surface.** Then trained nurse who used to perform blood collection procedure would disinfect the skin according to the steps as outlined in flowchart 1. At the end of the disinfection, after the “venipuncture site” was air dried, another culture plate was applied with a contact time of 10 seconds (after disinfection).

Microbiological methods and neutralizers

In part one of the study, commercial TSA (Tryptone Soya Agar) standard contact plate with neutralizers (0.3% Tween 80, 0.1% lecithin) (Oxoid, Germany) was used whereas in part two, an in-house TSA contact plate was further prepared which contained the

following neutralizers (3% Tween 80, 0.3% lecithin, 0.1% histidine, 0.5% sodium thiosulphate, 3% saponin, 1% ether sulphate). **The neutralizers were reported to have better neutralization effect on chlorhexidine [26] but non-toxic to bacteria such as *S. aureus*, *S. epidermidis*, *Micrococcus luteus*, *Corynebacterium jeikeium* and *E coli* [26, 27].** Following applications, the plates were immediately incubated at 37°C for 24 hours. Afterwards, the growth on the plates was photographed and colonies were counted manually by using the ImageTool software (version 3.0, University of Texas Health Science Center, San Antonio, Texas). The disinfectant assignments of the subjects were blinded to the research assistant who carried out the plate counting. As quality control, 10% of the images were randomly selected for a second count by the same research assistant. Scatterplot and correlation of determination (R Square) analysis demonstrated that there was high correlation between the first and second colony counts.

Sample size and statistical analysis

Sample size and power were determined by Statmate (GraphPad Software Inc, CA, USA). For part one of the study, with a baseline of 85% no growth after disinfection, a sample size of at least 80 per group would be required to detect a difference of 12% or 0.36 log with 80% power at 5% level of significance. As CHX/IPA-1 and CHX/IPA-2

was compared in part two study, no more than 10% difference was expected.

Therefore, sample size of 160 would be required for 80% power at 5% level of significance.

Descriptive statistics was performed and a paired student t-test using Prism version 5.03 was used (GraphPad Software Inc, CA, USA) was employed to determine any statistical difference. A *P* value of < 0.05 was denoted as statistically significant.

Results

For the part one of the study, 81 subjects were enrolled to be disinfected by PI/IPA and 85 were tested with CHX/IPA-1. Their baseline bacterial counts in both arms were comparable. After disinfection, both disinfectants could achieve significant bacterial reduction. As shown in Figure 1, the bacterial colonies per plate in log scale decreased from $2.70 \log_{10} \pm 0.42$ to $0.46 \log_{10} \pm 0.61$ in PI/IPA group whereas for CHX/IPX-1, it dropped from $2.54 \log_{10} \pm 0.76 \log_{10}$ to $0.02 \log_{10} \pm 0.11$. In fact, 50 out of 81 arms (61.7%) in PI/IPA group had residual bacterial colonies whereas only 5 out of 85 arms (5.9%) in CHX/IPA-1 group were found to have bacteria ($P < 0.001$) (Table 1).

For the part two of the study with 160 subjects participated, the pre-disinfection baseline bacterial count per plate in log scale for either arms assigned to CHX/IPA-1 and CHX/IPA-2 were $2.08 \log_{10} \pm 0.74$ and $2.08 \log_{10} \pm 0.75$, respectively (Figure 2 upper panel, $P = 0.86$). Upon disinfection, in the CHX/IPA-1 side, the bacterial count per plate dropped to $0.16 \log_{10} \pm 0.32$ whereas it was $0.23 \log_{10} \pm 0.4$ in the CHX/IPA-2 side (Figure 2 lower panel, $P = 0.07$). 42.5% (68/160) of arm after CHX/IPA-1 had residual bacterial count detected whereas 49.4% (79/160) of arm after CHX/IPA-2 had residual bacterial count detected ($P = 0.26$) (Table 1).

Discussion

Skin disinfection has been found to be a crucial step in minimization of bacterial contamination during blood donation [7, 23, 28, 29]. Therefore, a combination of best skin disinfectant and procedure should be identified and practiced in the daily blood collection procedure. The design of present study was influenced by the two evaluation studies by McDonald *et al* [21] and Ramirez-Arcos *et al* [24] in blood donation setting. Both of them showed one stage application of combined formulation of 2% chlorhexidine gluconate and 70% isopropyl alcohol (CHX/IPA) as skin disinfectant were effective. As our blood center had been using two stages application by 10% povidone-iodine/70% isopropyl alcohol (PI/IPA) [29] together with a long history of routine bacterial surveillance programme [30, 31], the CHX/IPA if proven to be effective in local setting would be advantageous because of the simplicity of application. Therefore, it would be necessary to first compare the efficacy of CHX/IPA versus PI/IPA in an evaluation environment.

From the results of part one of the study, it appeared that CHX/IPA was significantly better in lowering lower residual bacterial count than PI/IPA which was consistent to the findings from two evaluation studies [21, 24]. However, we noticed that the subjects' skin after using CHX/IPA appeared to be sticky suggestive of residual chlorhexidine. In retrospect, it is known from medical literature that this

non-volatile residual chlorhexidine could continue to exert antimicrobial effect on the skin and on the culture plate [32]. It might confer advantage over PI/IPA. Besides, the commercial standard contact plate (with Tween/Lecithin) used in this part of study was found to be unable to neutralize residual chlorhexidine [26, 27]. We therefore, argued that this contributed to pitfalls in the efficacy tests and should be properly addressed. To address this, the part two study was so designed to use a contact plate that could neutralize any residual chlorhexidine during sampling before culture. Though we acknowledged that direct comparison with PI/IPA could not be made, we observed that the residual bacterial colonies counts after applying CHX/IPA-1 or CHX/IPA-2 were still lower than that obtained from PI/IPA in part one of the study. Therefore, we believe that CHX/IPA would be a very effective skin disinfectant in blood donation but a proof may require further study using properly designed contact plates for direct comparison between PI/IPA and CHX/IPA.

When operation logistic was looked into, though about the same duration was required in applying PI/IPA or CHX/IPA to the skin for disinfection, a single application might result in an overall time reduction because of the elimination of opening up two packages for the whole procedure. Moreover, the swabstick was easier to manipulate by operator.

The present study was limited by not being able to have a direct comparison of PI and CHX skin disinfectants at the same time by using culture plates with their specific neutralizers. Besides, though no occurrence of allergy was observed in our small scale of evaluation study, caution must therefore, be exercised in routine application as other studies have suggested a higher rate of mild but self-limiting skin allergy in blood donors [25].

In summary, the present study demonstrates that one stage application of 2% chlorhexidine gluconate/70% isopropyl alcohol formulation (CHX/IPA) could replace the currently used sequential application of 10% povidone iodine and 70% isopropyl alcohol in pre-donation skin disinfection.

Acknowledgement:

B.K.L. So, C.C.Y. Chu, J.N.S. Leung and I.Y.M. Lee were responsible for the volunteers recruitment and performed the disinfection on the volunteers. K.H. Chow prepared the in-house contact plate, performed the culture and counted the residual bacterial colonies. C.K. Lee and P.L. Ho designed on the study and wrote the manuscript with B.K.L. So. C.K. Lin critically reviewed the manuscript

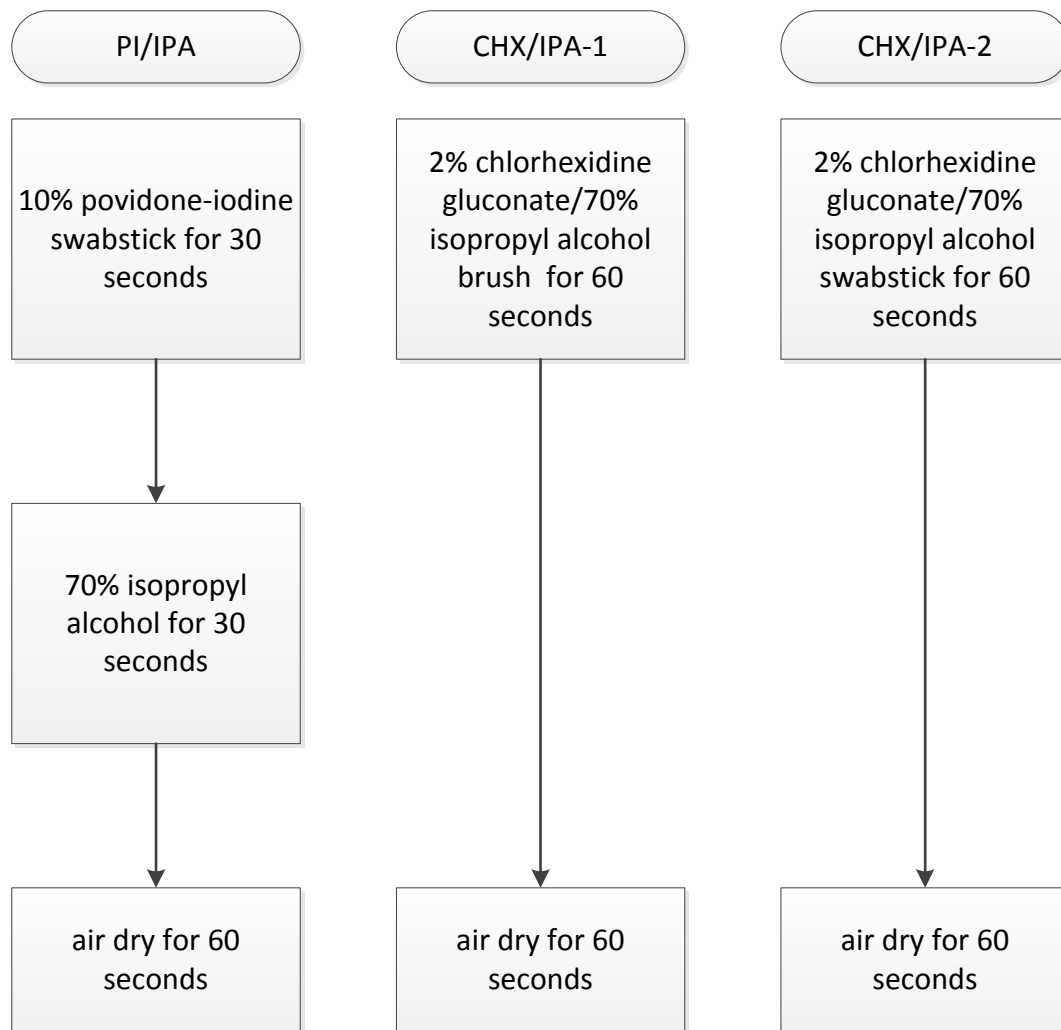
Reference:

- 1 Wagner S: Transfusion-related bacterial sepsis. *Curr Opin Hematol.* 1997;**4**: 464-9.
- 2 Wagner SJ, Friedman LI, Dodd RY: Transfusion-associated bacterial sepsis. *Clin Microbiol Rev.* 1994;**7**: 290-302.
- 3 Dave J, Brett M, MacLennan S, Shields M: Sepsis associated with blood transfusion. *Lancet.* 1996;**347**: 1773.
- 4 Chiu EK, Yuen KY, Lie AK, Liang R, Lau YL, Lee AC, Kwong YL, Wong S, Ng MH, Chan TK: A prospective study of symptomatic bacteremia following platelet transfusion and of its management. *Transfusion.* 1994;**34**: 950-4.
- 5 Zaza S, Tokars JI, Yomtovian R, Hirschler NV, Jacobs MR, Lazarus HM, Goodnough LT, Bland LA, Arduino MJ, Jarvis WR: Bacterial contamination of platelets at a university hospital: increased identification due to intensified surveillance. *Infect Control Hosp Epidemiol.* 1994;**15**: 82-7.
- 6 Lee CK, Ho PL, Chan NK, Mak A, Hong J, Lin CK: Impact of donor arm skin disinfection on the bacterial contamination rate of platelet concentrates. *Vox Sang.* 2002;**83**: 204-8.
- 7 McDonald CP, Lowe P, Roy A, Robbins S, Hartley S, Harrison JF, Slopecki A, Verlander N, Barbara JA: Evaluation of donor arm disinfection techniques. *Vox Sang.* 2001;**80**: 135-41.
- 8 de Korte D, Curvers J, de Kort WL, Hoekstra T, van der Poel CL, Beckers EA, Marcelis JH: Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands. *Transfusion.* 2006;**46**: 476-85.
- 9 de Korte D, Marcelis JH, Verhoeven AJ, Soeterboek AM: Diversion of first blood volume results in a reduction of bacterial contamination for whole-blood collections. *Vox Sang.* 2002;**83**: 13-6.
- 10 McDonald CP, Roy A, Mahajan P, Smith R, Charlett A, Barbara JA: Relative values of the interventions of diversion and improved donor-arm disinfection to reduce the bacterial risk from blood transfusion. *Vox Sang.* 2004;**86**: 178-82.
- 11 Wagner SJ, Robinette D, Friedman LI, Miripol J: Diversion of initial blood flow to prevent whole-blood contamination by skin surface bacteria: an in vitro model. *Transfusion.* 2000;**40**: 335-8.
- 12 International Forum: 1. *Vox Sang.* 2003;**85**: 224-5.
- 13 Liu HW, Yuen KY, Cheng TS, Lee KB, Chua EK, Ho PL, Lin CK: Reduction of platelet transfusion-associated sepsis by short-term bacterial culture. *Vox Sang.* 1999;**77**: 1-5.

- 14 Brecher ME, Holland PV, Pineda AA, Tegtmeier GE, Yomtovian R: Growth of bacteria in inoculated platelets: implications for bacteria detection and the extension of platelet storage. *Transfusion*. 2000;**40**: 1308-12.
- 15 AuBuchon JP, Herschel L, Roger J, Taylor H, Whitley P, Li J, Edrich R, Goodrich RP: Efficacy of apheresis platelets treated with riboflavin and ultraviolet light for pathogen reduction. *Transfusion*. 2005;**45**: 1335-41.
- 16 Ruane PH, Edrich R, Gampp D, Keil SD, Leonard RL, Goodrich RP: Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. *Transfusion*. 2004;**44**: 877-85.
- 17 Goodrich RP, Gilmour D, Hovenga N, Keil SD: A laboratory comparison of pathogen reduction technology treatment and culture of platelet products for addressing bacterial contamination concerns. *Transfusion*. 2009;**49**: 1205-16.
- 18 Janssen MP, van der Poel CL, Buskens E, Bonneux L, Bonsel GJ, van Hout BA: Costs and benefits of bacterial culturing and pathogen reduction in the Netherlands. *Transfusion*. 2006;**46**: 956-65.
- 19 Adams D, Quayum M, Worthington T, Lambert P, Elliott T: Evaluation of a 2% chlorhexidine gluconate in 70% isopropyl alcohol skin disinfectant. *J Hosp Infect*. 2005;**61**: 287-90.
- 20 Hibbard JS, Mulberry GK, Brady AR: A clinical study comparing the skin antisepsis and safety of Chloraprep, 70% isopropyl alcohol, and 2% aqueous chlorhexidine. *J Infus Nurs*. 2002;**25**: 244-9.
- 21 McDonald C, McGuane S, Thomas J, Hartley S, Robbins S, Roy A, Verlander N, Barbara J: A novel rapid and effective donor arm disinfection method. *Transfusion*. 2010;**50**: 53-8.
- 22 Small H, Adams D, Casey AL, Crosby CT, Lambert PA, Elliott T: Efficacy of adding 2% (w/v) chlorhexidine gluconate to 70% (v/v) isopropyl alcohol for skin disinfection prior to peripheral venous cannulation. *Infect Control Hosp Epidemiol*. 2008;**29**: 963-5.
- 23 Wong PY, Colville VL, White V, Walker HM, Morris RA, Microbial C, Infection Control Subcommittee ARCBS: Validation and assessment of a blood-donor arm disinfectant containing chlorhexidine and alcohol. *Transfusion*. 2004;**44**: 1238-42.
- 24 Ramirez-Arcos S, Goldman M: Skin disinfection methods: prospective evaluation and postimplementation results. *Transfusion*. 2010;**50**: 59-64.
- 25 Benjamin RJ, Dy B, Warren R, Lischka M, Eder AF: Skin disinfection with a single-step 2% chlorhexidine swab is more effective than a two-step povidone-iodine method in preventing bacterial contamination of apheresis platelets. *Transfusion*. 2011;**51**: 531-8.

- 26 Kampf G: What is left to justify the use of chlorhexidine in hand hygiene? *J Hosp Infect.* 2008;**70 Suppl 1**: 27-34.
- 27 Reichel M, Heisig P, Kampf G: Pitfalls in efficacy testing--how important is the validation of neutralization of chlorhexidine digluconate? *Annals of clinical microbiology and antimicrobials.* 2008;**7**: 20.
- 28 Goldman M, Roy G, Frechette N, Decary F, Massicotte L, Delage G: Evaluation of donor skin disinfection methods. *Transfusion.* 1997;**37**: 309-12.
- 29 Lee CK, Ho PL, Chan NK, Mak A, Hong J, Lin CK: Impact of donor arm skin disinfection on the bacterial contamination rate of platelet concentrates. *Vox Sanguinis.* 2002;**83**: 204-8.
- 30 Lee CK, Wong HK, Ho PL, Tsoi WC, Lee KY, Tsui GT, Chua E, Leung JN, Lin CK: Significant bacterial contamination risk reduction with the use of diversion pouch. *Transfus Med.* 2012;**22**: 404-8.
- 31 Liu H-w, Yuen K-y, Cheng TS-y, Lee K-b, Chua EK-m, Ho P-l, Lin C-k: Reduction of Platelet Transfusion Associated Sepsis by Short-Term Bacterial Culture. *Vox Sanguinis.* 1999;**77**: 1-5.
- 32 Kampf G, Shaffer M, Hunte C: Insufficient neutralization in testing a chlorhexidine-containing ethanol-based hand rub can result in a false positive efficacy assessment. *BMC infectious diseases.* 2005;**5**: 48.

Flowchart 1 showed the steps used for disinfection by three disinfectants.



^a PI/IPA, 10% povidone-iodine swab stick (Professional Disposables International Inc, NY, US) and 70% isopropyl alcohol swab (Professional Disposables International Inc, NY, US); Both CHX/IPA-1 and CHX/IPA-2 contained 2% chlorhexidine gluconate/70% isopropyl alcohol. CHX/IPA-1, ChloraPrep® 3 ml brush (Cardinal Health Inc, KS, US) CHX/IPA-2, SOLU-I.V. swabstick (SOLUMED, 3M Canada Company, Ontario, Canada).

Table 1. Comparison of the effectiveness of three different skin disinfection methods and recover by contact plates with two different mixtures of neutralizing agents.

Disinfection method ^a	Neutralizing agents in culture media	% with growth (No/total)	P
Part 1			
PI/IPA	0.3% Tween 80, 0.1% Lecithin	61.7% (50/81)	<0.001
CHX/IPA-1	0.3% Tween 80, 0.1% Lecithin	5.9% (5/85)	
Part 2			
CHX/IPA-1	TLHThSEt ^b	42.5% (68/160)	0.26
CHX/IPA-2	TLHThSEt	49.4% (79/160)	

^a See footnotes of flowchart 1 for the three disinfection methods.

^bTLHThSEt, 3% Tween 80, 0.3% lecithin, 0.1% histidine, 0.5% sodium thiosulphate, 3% saponin, 1% ether sulphate [26].

Figure 1: showed the bacterial counts before and after applying PI/IPA and CHX/IPA-1

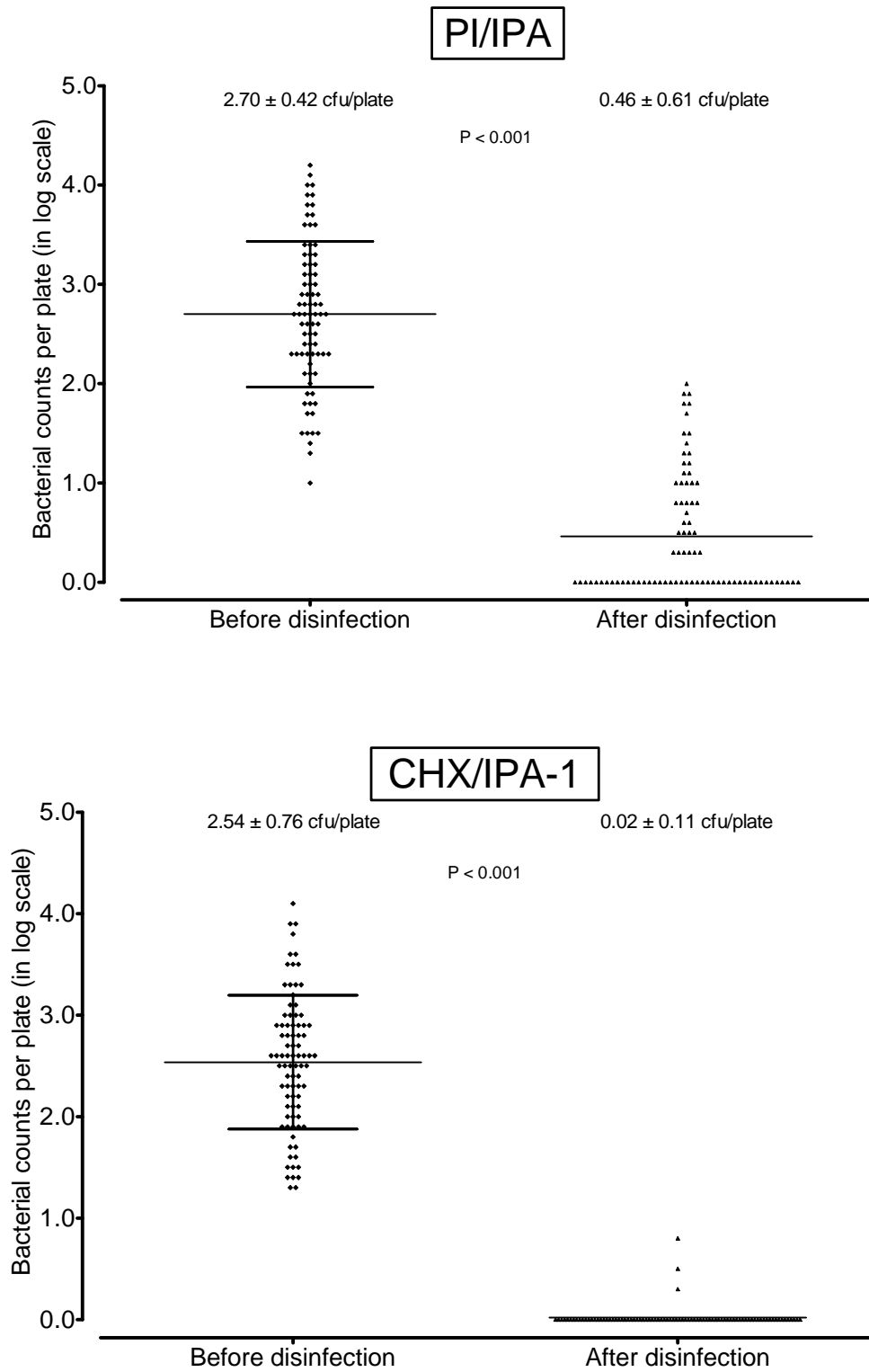


Figure 2 showed the bacterial counts before and after applying CHX/IPA-1 and CHX/IPA-2.

