

## The Comparison of Autoclave, Povidone Iodine 10%, and Boiling toward Radiological Union Rates and Bacterial Culture

Muhammad Ihsan,<sup>1</sup> Aryadi Kurniawan,<sup>2</sup> Marcel Prasetyo,<sup>3</sup> Ariyani Kiranasari<sup>4</sup>

<sup>1</sup> Residency Program of Orthopaedic and Traumatology, Faculty of Medicine, Universitas Indonesia  
Cipto Mangunkusumo Hospital

<sup>2</sup> Pediatric Consultant, Orthopaedic and Traumatology Specialist, Faculty of Medicine, Universitas Indonesia  
Cipto Mangunkusumo Hospital

<sup>3</sup> Senior Radiologic Consultant Orthopaedic and Traumatology, Faculty of Medicine, Universitas Indonesia  
Cipto Mangunkusumo Hospital

<sup>4</sup> Staff of Microbiologic Department, Faculty of Medicine, Universitas Indonesia

### ABSTRACT

**Introduction.** There are some controversies about the appropriate management of contaminated bone graft. The purpose of this study was to determine the which one is the most optimal method of decontaminating bone from which often used. Regarding to radiological union rates and microbiological culture.

**Materials and methods.** Twenty eight samples of femur were discarded from 28 male Sprague dawley rats divided into 4 groups of treatment. The bone samples were uniformly contaminated by broth containing *Staphylococcus aureus*, except the *Saline* group. The bone samples in each group underwent four different decontamination procedures: Normal *Saline*, autoclave, *Povidone iodine* 10%, and boiling, and reimplanted with *Kirchner wire* fixation.

**Results.** After 8 weeks reimplantation, Radiological examination showed that povidone iodine had higher radiological union rate and lower infection rate compared autoclave, boiling, and normal *saline*. There was no statistically significant difference in radiological union rate and bone graft infection rate among autoclave, *povidone iodine*, and boiling, except between *povidone iodine* and *saline* group.

**Conclusions.** Decontamination method should have minimal deleterious effect to cell viability and no infection risk. In this study, we found an easily accessible and clinically relevant method of decontaminating contaminated bone by using *povidone iodine* 10%. Decontamination of cortical bone sample by *povidone iodine* 10% offers the best balance between lower infection risk of contaminated bone and better union rate.

**Key words:** decontamination method, reimplantation, radiological union, infection risk.

Corresponding authors:  
Muhammad Ihsan, MD  
Jl. HR Subrantas Gg Sepakat No. 65, Panam Pekanbaru  
Riau 28294  
HP : 0813 65435965  
Email : doktermihsan@yahoo.co.id

## Perbandingan *Autoclave*, *Povidone Iodine 10%*, dan *Boiling* terhadap Nilai Penyatuan Radiologis dan Kualitatif Kultur Bakteri

### ABSTRAK

**Pendahuluan.** Ada banyak kontroversi tentang tata laksana fragmen tulang yang terkontaminasi. Tujuan penelitian ini adalah untuk menentukan metode dekontaminasi mana yang paling optimal diantara yang sering kita gunakan berdasarkan penyatuan radiologis dan mikrobiologi.

**Bahan dan cara kerja.** Dua puluh delapan fragmen tulang paha tikus putih Sprague dawley kemudian dikelompokkan menjadi 4 grup perlakuan. Tiap-tiap Fragmen tulang tersebut mendapat kontaminasi *Staphylococcus aureus*, kecuali grup saline. Kemudian tulang dari tiap grup didekontaminasi dengan *saline*, *Autoclave*, *povidone iodine 10%* dan *Boiling*. Kemudian direimplantasi dengan menggunakan *K wire*.

**Hasil.** Setelah 8 minggu reimplantasi, pemeriksaan radiologi menunjukkan hasil bahwa grup povidone iodine memberikan hasil penyatuan radiologis dan resiko infeksi yang rendah dibanding *Autoclave*, *Boiling*, dan *saline*. Tidak dijumpai perbedaan statistik yang bermakna diantara grup *Autoclave*, *povidone iodine*, dan *Boiling* kecuali antara grup *povidone iodine* dengan *saline*.

**Simpulan.** Metode sterilisasi yang ideal seharusnya tidak merusak viabilitas tulang dan dengan resiko infeksi yang rendah. Pada studi ini kami menemukan metode sterilisasi yang mudah dan efektif secara klinis yaitu dengan *povidone iodine*. Dekontaminasi fragmen tulang yang terkontaminasi dengan povidone iodine 10% menghasilkan resiko infeksi yang rendah dan penyatuan radiologis yang lebih baik.

**Kata kunci:** metode dekontaminasi, reimplantasi, penyatuan radiologis, resiko infeksi

### Introduction

Fracture is one of major health problem in the world.<sup>1,2</sup> In USA alone, patients visit doctor due to fractures reach 11,4 millions each year, whilst about one million of it admitted to ward.<sup>3</sup> The risk of a fracture is happen to a person for his/her entire life is around 50%. In Scotland from 2007-2008, incidence of fracture reaches 13.7 from 1000 people. Around 2.6% of them are open fractures.<sup>2</sup>

In open fracture there is a connection between bone fragment and its surrounding soft tissue to outside environment, thus contaminated with bacteria leading to infection.<sup>1,2</sup> One of the principal management in dealing with open fracture is prompt debridement and wound irrigation.<sup>1,2,4,5</sup>

In order to achieve adequate debridement, doctor need to excise all non vital tissue. Small bone fragments which no longer attached to soft tissue and suspectedly contaminated need to be removed because it would be difficult to clean it adequately. Aside from those, the use of allograft to fill in bone defect carries risks of viral transmission (Creutzfeld-Jacob disease, hepatitis C,

AIDS, and others) and immunological reaction within host body.<sup>3,5,6</sup>

From literature we could find several recommendations on decontaminating bonegraft in operating room. Ellis recommended the use of 0,6 N HCl for 60 minutes then submerged into ethyl alcohol for about 10 minutes. Some authors recommended decontaminatin with *povidone iodine scrub* for 10 minutes followed by autoclave process, which proven to have sterilized dirt contaminated bone graft. Other study showed that scrubbing with Chlorhexidine Gluconate then soaked in 50.000 units of Polimiksin B is adequate to eliminate contaminated bacteria.<sup>6</sup> Others found that soaking contaminated bone fragment in 70% ethanol for 8 hours resulting in complete sterilization and also reducing its osteoinduction capability.<sup>7-10</sup>

Autoclave has been used to sterilize autograft bone for reconstruction purpose since 50 years ago in orthopaedy or neurosurgery. Authors reported that bone fragment which had been processed by autoclave was placed back side-by-side with living bone, thus it will gradually

repopulate with osteocyte. Previous event could happen because there are revascularization, resorption and new bone formation.<sup>7,8</sup>

In cases there aren't any decontamination devices available we need alternative method to decontaminate the bone. One of which is High Level Decontamination process, this method is acceptable because it could kill almost all of microorganism (including vegetataif bac-tery, M. tuberculosis, yeast and virus) excluding few endospores. The methods is very simple, we process the bone with submerging it into boiled water at least for 20 minutes.<sup>11,12</sup> Schultke<sup>13</sup> stated that the boiling process for 30 minutes could eradicate pathogenic microorganism on the contaminated bone, but he didn't assess its bone healing capability after boiling.

Many literatures stated that *povidone iodine* 10% is effective to decontaminate contaminated bone. It is also stated that *povidone iodine* is effective to decontaminate contaminated bone. But, all of those three literature did its study in *in vitro*, so that end result from the bone that subjected to decontamination process is unknown.<sup>6,14,15</sup>

From that, we can see that further study is necessary, a study focusing on ideal decontamination method which could effectively sterilize the bone fragment, but doesn't tamper with its bone healing capability.

From previous background we could formulate the problems as whether there is a different effect in auto-clave, *povidone iodine* and boiling to radiologic union score and quantitative bacterial culture after reimplantat-ing contaminated bone fragment.

This study hypothesis is there is a difference effect in autoclave, *povidone iodine*, and boiling to to radiologic union score and quantitative bacterial culture after reimplantating contaminated bone fragment.

This study aim is to assess which decontamination methods that the best fit for decontaminating contami-nated bone fragment which would be used for recon-struction. In specific, the study is about to assess the dif-ference between autoclave, *povidone iodine* and boiling method on radiologic union rate of reimplanted bone fragment and bacterial culture of the reimplanted bone fragment.

Results from this study could provide as data to find out autoclave, *povidone iodine* 10% and boiling effec-tiveness on decontaminating contaminated bone frag-ment.

## Materials and methods

The study design was experimental study. This study sample population was *Sprague dawley* white rat with

Table 1. Lane and Sandhu Criteria of Radiologic Bone Union Score

Criteria	Scores
There is no bone healing	0
Callus formation	1
New bone formation	2
Osteotomy line almost dissappear	3
Complete bone healing	4

11 - 12 weeks of age 250-300 grams of weight. It was bred and kept at laboratory of animal subject BALIT-BANGKES RI from March through May 2012.

This study had 4 groups; group C (control), group B (*povidone iodine*), group O (autoclave) and group R (Boiling). Sample for each group was randomly selected. Estimation of sample size for each group was determined by Federer formulation resulting in six white rats. To anticipate drop out cases, each group were added one extra rat, thus each group had seven white rats. All samples met the study criteria.

Evaluation method was done radiologically and mi-crobiologically. Radiologic evaluation was based on ra-diologic union scores by Lane and Sandhu (table 1).<sup>20</sup> Assessment was performed by radiologic specialist. On the other hand, micobiological evaluation was based on culture result of *Staphylococcus* bacterly on blood agar media which then identified through Gram staining and confirmed by MSA (*manitol salt agar*). This assessment was performed by microbiology expert.

## Results

After 8 weeks, each subject underwent radiologic and micobiologic assessment. The result was shown in table 2.

*Radiologic Assesment.* From table 2 we could find that there was a significant difference on radiological union scores among all treatment groups compared to control group. From table 3 we could see that the p value is 0.033. The highest radiologic union score was find at group B (*povidone iodine*) with median of 4.0 (3.0-6.0), whilst the lowest score was at control group with median of 2.0 (0.0 – 2.0).

Table 4 showed paired comparison between group B and group C, and the p value is 0.0018. Although after correction with Dunn method for multiple comparison (post hoc analysis) the p value become 0.052 which sta-tistically speaking the difference is less significant.

Table 2. Result of radiologic and microbiologic assessment

	Culture	Radio- logic Union Scores		Culture	Radiologic Union Scores
<b>Group C (Control Group)</b>			<b>Group B (Povidone Iodine)</b>		
C1	+	2	B1	-	6
C2	+	2	B2	-	5
C3	+	0	B3	-	3
C4	+	0	B4	+	2
C5	+	5	B5	-	4
C6	+	2	B6	-	2
C7	+	2	B7	-	6
<b>Group R (Boiling)</b>			<b>Group O (autoclave)</b>		
	Culture	Radio- logic Union Scores		Culture	Radiologic Union Scores
R1	+	2	O1	+	1
R2	+	3	O2	+	3
R3	+	2	O3	-	1
R4	-	3	O4	+	1
R5	+	2	O5	+	2
R6	-	6	O6	-	2
R7	-	2	O7	-	5

*Culture Bactery Assessment.* Table 5 showed that there is a significant difference (calculated with Fischer test,  $p=0.011$ ) among treatment groups compared to control group. The highest rate of positive bacterial culture was control group with 100% rate. The lowest rate of positive bacterial culture was Group B (*Povidone iodine*). While group R and group O showed 57.1% positive culture rate.

On table 6 we could see that the significant difference only between group C (Control) and group B (*Povidone iodine*) with  $p$  value of 0.005.

## Discussions

Bone fragment which had been processed through autoclave require longer time to heal.<sup>17</sup> Autoclave cause reduction of osteoinduction capability and mechanical strength of the bone. Supplementation of *allogenic bone matrix* on osteotomized area plus osteosynthesis would increased its incorporation and strength.<sup>16</sup> Autoclave causes edema and matrix dissolution through denaturation. This is one of the reason for the bone to have a decrease on biologic potential.

Comparison between group O and group B showed no significant difference statistically ( $p=0.092$ ). Nonetheless, in group B most of the subject showed radiologic bone formation (score 2) there is even one subject showed a disappearing osteotomy line (score 3). While all of the subject in group O (autoclave process) only showed callus formation (score 1). It showed that even statistically non significant but clinically it has significant effect. Hence, group B (*povidone iodine*) have better bone healing process when compared to group O. Decontamination of bone fragment with *povidone iodine* gives advantages on complete decontamination and better preservation on cell viability. They also suggested application of *povidone iodine* 10% for 15 minutes as a method of decontamination of contaminated bone fragment before reimplantation.<sup>15</sup> Yaman *et al*<sup>14</sup> found that there were active osteoblast, normal osteocyte and regular *Harvesian canal* on histologic evaluation one white rat's bone who previously underwent decontamination with *povidone iodine* 10% for 15 minutes.

Similar as group O (autoclave), group R (Boiling) showed relatively similar radiologic result, lower score

Table 3. Comparison of radiological union score between all of the treatment group compared to control group.

	<b>Radiologic Union Scores</b>	<b>P value*</b>
Group C (Control)	2.0 (0.0-2.0)	0.033
Group B ( <i>Povidone iodine</i> )	4.0 (3.0-6.0)	
Group R (Boiling)	2.0 (2.0-3.0)	
Group O (Autoclave)	2.0 (1.0-3.0)	

\*Data is presented in median with inter quartile range. P value was calculated using nonparametric test Kruskal-Wallis.

Table 4. Paired comparison between two group for radiologic union scores

	<b>p value*</b>	<b>Corrected p value†</b>
Control vs. <i>Povidone iodine</i>	0.018	0.052
Control vs. Boiling	0.136	1.000
Control vs. Autoclave	0.789	1.000
<i>Povidone iodine</i> vs. Boiling	0.097	1.000
<i>Povidone iodine</i> vs. Autoclave	0.027	0.092
Boiling vs. Autoclave	0.202	1.000

\*p value was calculated with Mann-Whitney U nonparametric test for two groups.

†p value was calculated with Kruskal-Wallis nonparametric test for more than two groups, followed by post hoc analysis with Dunn method for correction of multiple comparison.

Table 5. Result of Bacterial Culture for each group

	<b>Culture</b>		<b>P value</b>
	<b>Positive</b>	<b>Negative</b>	
Control	7 (100.0)	0 (0.0)	0.011
<i>Povidone iodine</i>	1 (14.3)	6 (85.7)	
Boiling	4 (57.1)	3 (42.9)	
Autoclave	4 (57.1)	3 (42.9)	

Data is presented in frequensi (proportional percentage)

P value was calculated using Fischer test

Table 6. Perbandingan berpasangan antar dua-kelompok-tuk hasil culture bakteri.

	P value
<b>Control vs Povidone iodine</b>	<b>0.005</b>
Control vs Boiling	0.192
Control vs Autoclave	0.192
Povidone iodine vs Boiling	0.266
Povidone iodine vs Autoclave	0.266
Boiling vs Autoclave	1.000

P value calculated with Fischer test.

compared to group B (*povidone iodine*). Knaepler et al stated that temperature of 80° C through 134° C would reducing bone healing capability because the bone graft would be difficult to incorporate to its host.<sup>17</sup>

In this study we could see that exposure to autoclave process and boiling process resulting in negative effect on bone graft ability to incorporate. We think that decontamination method should have the ability to eradicate pathogenic microbacteria and the ability to preserve biological properties and mechanical properties of a bone graft.

We know that bone fragment from control group didn't contaminated with *Staphylococcus aureus*, only rinsed with NaCl 0,9% and then promptly reimplanted to its host without prior decontamination. This event is actually similar to open fracture, which operation procedure that would be done exposing the bone to outside environment and reimplanted without prior decontamination process. Yet, the result showed all subjects on control group were positive.

Comparison between group O (Autoclave) and group C (Control) showed a p value of 0,192 which means there isn't any significant difference statistically. Khattak MJ et al found that infection rate on patients of musculoskeletal tumor cases that underwent reimplantation of bone fragment after prior autoclave decontamination process is 5 out of 12. That thing could be because of wide incision technique or the length the operation.<sup>16</sup>

Bauer et al,<sup>8</sup> Hooe et al,<sup>14</sup> Yaman et al<sup>15</sup> stated that in their study, they all got a negative culture result from their bone fragment that had been decontaminated with autoclave. All studies did their microbiology examination promptly after decontamination with autoclave. On the other hand this study did it after 8 weeks of observational period. This showed that in our study did the examination to determine the long term infection risk to the bone which had been decontaminated and reimplanted.

Table 2 showed that 4 out of 7 (57.1%) subject from group O (autoclave) yield a positive culture result. Also on group R (boiling) yield the same result. Whilst the highest proportion is on group C (control) with 100% and the lowest is on group B (*povidone iodine*). This means that decontamination with *povidone iodine* 10% is better to lower the long term infection risk compared to autoclave or boiling method.

Possible cause that resulting to 57.1% positive rate on group O (autoclave) is autoclave negative effect on the bone fragment. Matsuno et al<sup>18</sup> in his study found high infection rate (259%) in long term follow up (around 1834 days) to his patient which had been underwent cranioplasty with autoclave autogenous bone graft. Those are possible because the bone structure would become lobulated after autoclave processes, so that it easily induced bacteria proliferation.

On the other hand, at group B (*povidone iodine*) there is only 14.3% positive culture result. This showed that soaking the bone fragment *povidone iodine* 10% for 20 minutes is better decontamination method compared to autoclave and boiling. This is also showed that *povidone iodine* 10% has lower long term infection rate that autoclave and boiling. Hooe and Steinberg stated that decontamination with *povidone iodine* 10% for 15 minutes on previously contaminated with *P.aeruginosa* and *S.aureus* is effective to eradicate those pathogens. Furthermore, they suggested to use *povidone iodine* 10% as a decontamination method for bone fragment because it does not damage bone histologic structure.<sup>8</sup>

The high proportion on positive culture result on group R (boiling) is correspondence on study from Schultke<sup>13</sup> which stated boiling process for 15 minutes on contaminated bone fragment is not effective to eradicate all of the pathogenic bacterial on it

## Conclusions

We found that there's no statistical difference among autoclave, *povidone iodine* and boiling as decontamination method regarding radiological bone union and bacterial culture on decontaminated bone fragment. Soaking decontaminated bone fragment with *povidone iodine* for 20 minutes gives better radiologic union and less long term infection risk compared to autoclave or boiling method.

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