

**Boiled water stored overnight is unsafe for consumption  
(conceptual note from *Sowarigpa*): a pilot prospective  
observational in-vitro analysis**

Jigme Singye<sup>1</sup>, Phuntsho Wangmo<sup>2</sup>

<sup>1</sup>Msc. Student, Faculty of Traditional Medicine, KGUMSB

<sup>2</sup> Sr. Laboratory technician, Lhuentse hospital

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**ABSTRACT**

**Background:** Irrespective of seasons or location, most of the people preserve boiled water for future use either in refrigerator or in room temperature, however medical text of *Sowarigpa* states that boiled water which is stored for a night is not safe for consumption. **Objectives:** To see the changes taken place in boiled water after 24 hours, 48 hours, 72 hours and 96 hours, which is stored at room temperature (19-28°C) and in refrigerator (2-8°C). **Methods:** An observational study was conducted at Lhuentse District Hospital lab in June 2017. Boiled water, which is stored at room temperature and 2-8°C were observed for 120 hours. During the course of investigation, the following parameters were tested, including ph of water, fecal coli-form, turbidity and growth of other colonies of organisms. **Result:** After 24 hours, the appearance of few colonies was observed in the boiled water (group A) that kept at room temperature and no colonies were found in the boiled water (group B) which is kept at 2-8°C. In 48 hours, growth of countable colonies was observed in both the sample and proportionately increased the colonies over the period of study. Potential of Hydrogen (pH) remained almost same thorough the study and turbidity was comparatively high in group A sample. **Conclusion:** the study illustrated that the boiled water after

24 hours becomes unsafe which is in alignment to the medical text of *Sowa Rigpa*.

**Keywords:** Boiled water; biochemical; colonies; fecal; organisms; sowarigpa.

## BACKGROUND

About 50-65% percent of the adult human body is made up of water. The percentage of water in infants is much higher, typically around 75-78% water, an average of 70% of human body is made up of water<sup>(1)</sup>, water is essential to all living beings, we need water for daily activities, and we need water to stay clean and healthy. People consume water either fresh/raw or boiled to quench their thirst.

There is no literature found in biomedicine stating boiled water left over a night is unsafe for consumption, however the literatures in *Sowa Rigpa* states that cool fresh water is said to have the beneficence against faint, exhaustion, hangover, dizziness, vomiting, disorder of blood and *Thripa*. Hot boiled water generates heat and help in digestion and metabolic activities. It instantly relieve hiccup, *Baed-Kan* disorder, distension of stomach, common cold and acute onset epidemic. Cooled boiled water is good for balancing *Thripa* disorder and does not aggravate *Baed-Kan*. However it becomes unsafe for consumption if it is left for a night<sup>(2)</sup>.

Therefore, an in-vitro analysis of water was conducted with extreme curiosity to see what physical or chemical change of the boiled has taken place when it is kept over a night. Self designed data recording sheet was used to keep the record of the variation observed during the entire investigation; ph value, turbidity, growth of other organisms and fecal coli formation.

**OBJECTIVE**

To observe what changes have taken place in boiled water stored at room temperature (19-28°C) and in refrigerator (2-8°C) after 24 hours, 48 hours, 72 hours, 96 hours and 120 hours?

**Setting**

Lhuentse Dzongkhag is bordered by Bumthang to the west, Trashiyangtse to the east, Mongar to the south and Tibet to the north. The Dzongkhag is considered one of the remotest and least developed in the country. With 3001 households, it has a total population of 15,395 consisting 7727 males and 7668 females (3). The district has eight Gewogs, 14 Basic Health Unit and a hospital and two traditional medicine units (4). This study was conducted in Lhuentse hospital in laboratory unit.

**Data variable****Water group**

Group A (Boiled water at room temperature)

Group B (Boiled water in the refrigerator)

Group C (Fresh tap water) for initial test to confirm the presence of pathogens.

Group D (Negative control, Distilled water)

Group E (Positive control, drain water)

**METHODS**

The study was an in-vitro observational study followed up to 120 hours. Boiled water, which is stored in a room temperature and refrigerator was tested for pH change, fecal coli formation, turbidity change and other bacterial growth every after 24 hours, 48 hours, 72 hours, 96 hours and 120 hours.

The analysis of water was conducted as per the standard protocol of the rural water supply plan (annexure I). Four liters of fresh tap water was collected from Lhuentse District Hospital and was divided into three groups A, B, and C. Group A and B was boiled for 100°C and stored in a disinfected container labeled as group A for boiled water which was kept in room temperature and as group B which was kept in the refrigerator, these two boiled water was investigated every after 24 hours, 48 hours, 72 hours, 96 and 120 hours with negative control group D(distilled water) and group E (drain water) as a positive control,

For initial testing, group A, B, C, D and group E was tested at zero hour, from next 24 hours, all groups were tested for the changes till 120 hours except group C water.

### **DATA ANALYSIS**

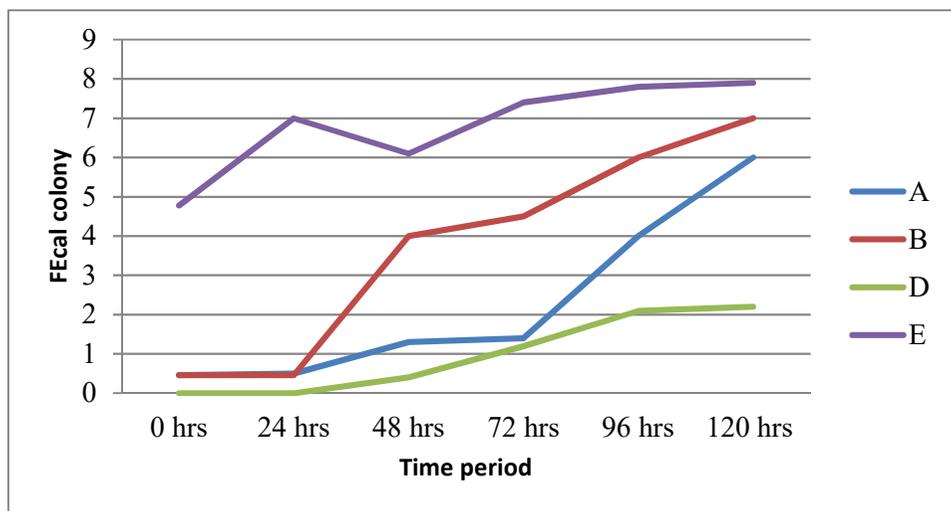
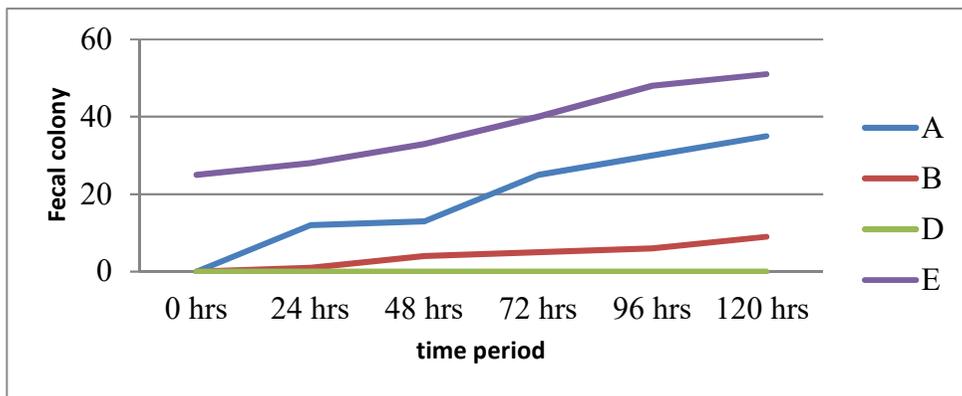
The variation of water in all groups over the period of study was visually observed. pH of water and turbidity was investigated with the help of pH reading equipments and turbidity reading machine. Fecal coli-form and growth of other bacteria was counted manually. The data was entered and analyzed in Microsoft excel worksheet.

### **RESULTS**

Despite scanty literature on the unsafe nature of boiled water in modern medicine, the finding of the study is in line with the statement of medical text of *Sowa Rigpa* “**boiled water left overnight is unsafe for consumption**”. The word “unsafe” means “not safe”, anything becomes normally unsafe when it is exposed to pathogenic organisms, the appearance of colonies in the sample water becomes unsafe for consumption. The development of colonies in group A (boiled water at room temperature) found to be proportionately increased from

the second day of observation till 120 hours. In group B, the growth of colonies of other organism was observed only from 48 hours and proportionately increased over the period of study. However the number of colonies visually seen was significantly higher in group A. (Figure 1). Potential of Hydrogen (pH) of the water remained almost same in all groups of water; however the turbidity of the water is comparatively high in group A over the time period (figure 2 &3).

**Figure 1: Colonies formed over the period of study.**



**Figure 2: Turbidity of the water over the period of study**

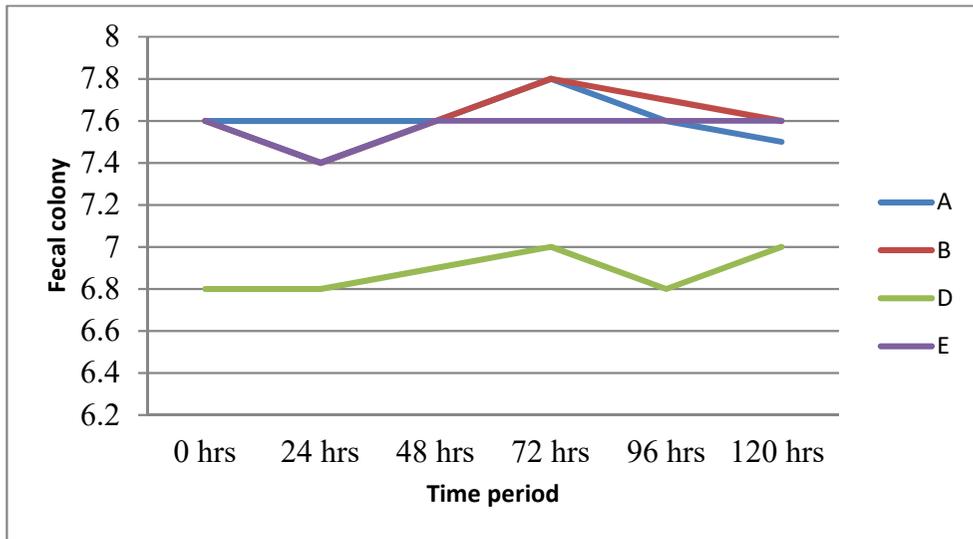
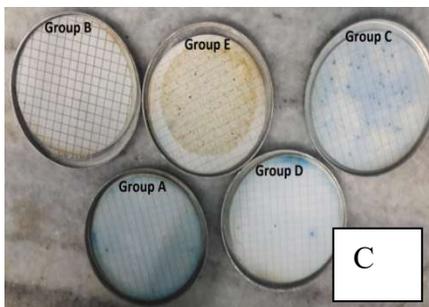


Figure 3: pH of water over the period of study





**Figure 4. A- Colonies formed in 24 hours, B- Colonies formed in 48 hours, C- Colonies formed in 72 hours, D- Colonies formed in 96 hours, E- Sample water**

### **Discussion / Conclusion**

The boiled water which is kept at the room temperature becomes significantly unsafe for consumption compared to the water kept in the refrigerator after 24 hours. The study also illustrated that even the water, which stored in the 2-8°C refrigerator becomes unsafe for consumption after 72 hours. However the appearance of colonies in the Petri dish needs to be isolated further for higher biochemical test for identification of organisms.

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