

# Growth of Streptococcus mutans in developer solutions with water supply and aquadest solvents

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## Growth of *Streptococcus mutans* in developer solutions with water supply and aquadest solvents

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### Abstract

**Background:** Radiographic processing involves intraoral film which contaminated with oral cavity that has *Streptococcus mutans* in it, then it can contaminate equipment in a dark room. Therefore, it can be the source to cross-infection. Developer solution made at Universitas Airlangga Radiology Clinic uses tap water from river in Surabaya as its solvent. The results of the tap water quality test shows various minerals such as manganese, zinc, and iron. Manganese, iron, and zinc are proven can influence the growth of *Streptococcus mutans* and biofilm development. This bacteria can contaminate the developer solution during the radiographic process and its growth can be affected by the mineral in the tap water as a developer solvent.

**Purpose:** To determine the growth of *Streptococcus mutans* in developer solution with water supply and aquadest as solvent and to know the differences between them.

**Method:** The concentrated developer was dissolved in the tap water and aquadest then routinely used at radiology clinic in dental hospital of Universitas Airlangga. Samples was taken before any films washed as control and sample of test taken 16 times each. Every sample can be taken after 8 films were washed. Control and test samples were diluted on BHIB media, planted in TYC media, and incubated for 2x24 hours. Bacteria were identified and then the number of colonies can be counted.

**Result:** There was growth of *Streptococcus mutans* as much as one colony in two samples of developer solution with a water supply solvent. There was no growth of *Streptococcus mutans* bacteria in the developer solution with aquadest solvent. There was no significant difference in growth of *Streptococcus mutans* bacteria in the solution of the developer of the water supply and the aquadest solvent.

**Conclusion:** The growth of *Streptococcus mutans* bacteria in the developer solution with a water supply solvent is more increasing than the aquadest solvent.

**Keywords:** developer solution, tap water, aquadest, *Streptococcus mutans*

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### INTRODUCTION

Treatment in the field of dentistry is generally supported by radiographic examinations. The examination is useful for establishing a diagnosis of a disease. An observational study with 30 sample questionnaires was given to dentists and the results were 73.33% of dentists using radiography as a supplementary examination (Ishaq, 2015).

Spread of infection can occur in the process of taking radiographic photographs through photo processing tools and materials used, because these tools and materials have the potential to become a place for growth of microorganisms (Santos, R.M.D., Santos, et al. 2012). During the film washing process, intraoral film radiographs, there is a chance of contamination of the patient's oral cavity fluid and microflora as well as the potential to contaminate equipment in the dark room. White and Glaze's study states that radiographers can

transfer microorganisms from the patient's oral cavity to radiographic equipment when making intraoral radiographic images and these microorganisms can survive on radiographic equipment for at least 48 hours (Fernandes, et al. 2013). Other studies have shown that the *Streptococcus* species occupies the third position after *Staphylococcus* and *Bacillus* bacteria which can contaminate radiographic films which is as much as 3.6% (Khalil, 2013). In other studies that tested the growth of five kinds of bacteria during the radiographic process, it was found that in the developer solution found growth of two dominant bacteria and one of them was *Streptococcus mutans*. In other studies, high concentrations of *Streptococcus mutans* contaminated

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in radiographic processing solutions can survive in developer and fixer solutions for up to two weeks. Despite technological advances, cross infection is still considered a risk in the field of dentistry. Not a few studies have also been directed at infection control and several researchers have conducted surveys of infection control and cross infection practices in the field of dentistry (Khalil, 2013).

Making developer solution requires water as a solvent for its components. The water supply (PDAM water) used comes from rivers in Surabaya as the solvent. Rivers in the Surabaya region get a lot of pollution from the surrounding environment, both from domestic waste and industrial waste. The worse the quality of river water in Surabaya, the drinking water raw material that will be used by the Regional Drinking Water Company (PDAM) will be increasingly polluted, thus the addition of chemicals in drinking water treatment will be more and more. (Priyono, Yuliani, & Sayekti, 2013; Indonesia, 2017). On the results of water quality testing tests for PDAM Ngagel installation, the water contains various mineral elements including ammonia, nitrite, nitrate, chloride, sulphate, iron, fluoride, chromium, cadmium, manganese, zinc, and copper.

Aquades water is distilled water with a permanent chemical form in the form of H<sub>2</sub>O. Aquades water is pure water, where biological contamination, organic and inorganic materials, heavy metals, and gases have been removed. This water does not have mineral elements as found in PDAM water. High concentrations of manganese, cadmium, and copper in saliva have been associated with increased caries prevalence. The increasing prevalence has led researchers to conduct study on the influence of mineral elements, especially manganese, on the bacterium *Streptococcus mutans* which is closely related to caries. Manganese is needed for detoxification of reactive oxygen species by most bacteria. The study of Arirachakaran et al. (2007) at concentrations of 50-100 µM manganese showed the maximum growth of *Streptococcus mutans*. The concentration of dissolved manganese in water generally ranges from 0.18 to 182 µM. In addition to manganese, study shows that the elements of iron and zinc in certain concentrations can affect the growth of *Streptococcus mutans*, i.e. iron can increase its growth by 2.8 times, while zinc can increase growth of two times (Aranha, et al. 1982). Iron can also increase the aggregation ability of *Streptococcus mutans* bacteria and the development of biofilms in the free phase (fluid phase) or in the adherent phase (Francesca, et al. 2004).

*Streptococcus mutans* bacteria is proven to be the most resistant bacterium against radiographic processing (Bachman, et al. 1990). This bacterium has the potential to contaminate developer solutions during radiographic photo processing and its growth can be influenced by mineral elements found in PDAM water as

developer solvents. Based on this background, then on this occasion the author wants to examine the growth of *Streptococcus mutans* bacteria in developer solutions with water supply solvent and aquades solvent.

## 6 MATERIALS AND METHODS

This study was a laboratory experimental research. This study has been approved by the ethical feasibility of this study has been given by the Health Research Ethical Clearance Commission (HRECC) Faculty of Dentistry, Universitas Airlangga (110 / HRECC.FODM / VII / 2018). The study was conducted at the Dental Radiology Poly Clinic at the Dental Radiology Poly Dental Hospital of Universitas Airlangga, Surabaya. The samples used were developer solutions with PDAM water and aquades water solvent, each of which amounted to 16 samples. Control samples were taken in the developer solution with the PDAM water solvent and aquades water solvent. Samples were taken and diluted on Brain Heart Infusion Broth (BHIB) media, then planted in Tryptone Yeast Cysteine (TYC) media to see the growth of the genus *Streptococcus* and incubated for 2x24 hours. The developer solution with each solvent is used routinely at the Dental Radiology Poly Clinic of RSGM Universitas Airlangga. Then, the samples were taken in the developer solution with PDAM water solvent and aquades water solvent each 16 times. In a study that used developer solution and was contaminated with *Streptococcus mutans*, *Streptococcus mutans* was obtained on the 8th film taking, so the treatment samples were taken after washing the film each multiple of 88. Samples with PDAM water solvents were labelled 1 and samples with aquades water solvents were given label 2 on each tube. The sample was diluted on BHIB media, then planted in TYC media to see the growth of the genus *Streptococcus* and incubated for 2x24 hours. TYC (Tryptone Yeast Cysteine) media was used to see the growth of *Streptococcus mutans* bacteria, each of which has white rounded colonies. *Streptococcus mutans* colonies were counted in CFU / ml.

## RESULTS

The results of the development and growth of the *Streptococcus mutans* bacteria were seen through two different developer dices. **Figure 1** showed the growth of bacteria in two samples of developer solution with PDAM water solvent. **Figure 2** showed the absence of bacterial growth in the sample developer solution with aquades water solvent.

The study data was processed and in **Table 1** showed the normality test data. The significance value obtained indicated that the data were not normally distributed. Then, a significance test was conducted between groups and the Mann Whitney Test to see differences between treatment groups.



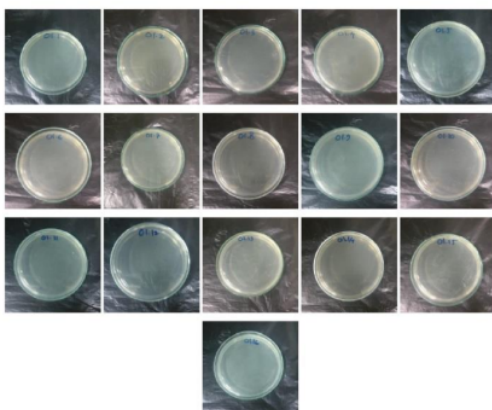


Fig. 1. Sample on water supply

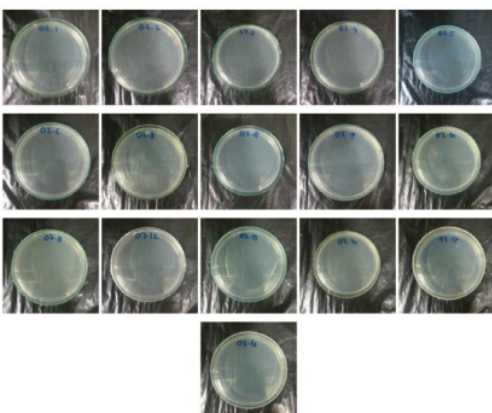


Fig. 2. Sample on aquadest water

Table 1. Normality test with Kolmogorov-Smirnov test

|                | Number of Sample | Mean  | Deviation Standard | Significance |
|----------------|------------------|-------|--------------------|--------------|
| PDAM water     | 16               | 0.125 | 0.34               | 0.00*        |
| Aquadest water | 16               | 0     | 0                  |              |

\* significant

Table 2. Different tests with the Mann Whitney test

|                | Number of Sample | Mean | Deviation Standard | Significance |
|----------------|------------------|------|--------------------|--------------|
| PDAM water     | 16               | 17.5 | 280                | 0.151*       |
| Aquadest water | 16               | 15.5 | 248                |              |

\* significant

Mann Whitney Test results obtained significance  $p = 0.151$ . The value of  $p > 0.05$  means that  $H_0$  was rejected and  $H_1$  was accepted, so it can be concluded that the number of Streptococcus mutans colonies in the PDAM solvent group does not have a significant difference with the number of Streptococcus mutans colonies in the Aquadest solvent group.

## DISCUSSION

The results of the developer solution with water supply solvent or with aquadest solvent prior to washing were not found the growth of Streptococcus mutans. Bacteria are not obtained because the developer solution has not been contaminated. In the treatment sample the results did not show any significant difference between the two solvents. The results of the developer solution with PDAM water solvent showed the growth of Streptococcus mutans bacteria in only two samples. While the results of the developer solution with aquadest water solvent showed no growth of Streptococcus mutans bacteria. Based on data analysis, although bacterial colonies can be found in two samples of developer solutions with PDAM water solvents, the growth does not show a significant difference in the growth of Streptococcus mutans bacteria between PDAM water solvents and aquadest water. PDAM water is not proven to have a major effect on increasing the growth of Streptococcus mutans bacteria in developer solutions.

The presence of Streptococcus mutans bacterial colonies in two sample developer solutions with PDAM water solvents can be caused by the condition of patients with different oral hygiene, so the bacterial concentrations in each washing are not the same. However, in other studies using bacterial concentrations of 108 to 1012 bacteria / ml, showed the survival of S. mutans in developer solution (Bachman, et al. 1990) Patients in both samples obtained by Streptococcus mutans probably had more concentrations of Streptococcus mutans bacteria than other patients, thus the bacteria could contaminate developer solution and can still survive the radiographic processing at the time of collection. Another factor is the lack of good infection control at the time of sampling. The possibility of the operator washing the film using gloves that come in contact with saliva thus there is contamination of Streptococcus mutans bacteria. In addition, the possibility of the two samples containing the growth of Streptococcus mutans colonies was conducted rapid sampling, thus the saliva of patients containing Streptococcus mutans was still present on the surface of the developer solution (Ahn, et al. 2008).

The absence of Streptococcus mutans colonies in the developer solution can be caused by a variety of factors. This is possible because the universal precaution in Poly Radiology RSGM Universitas Airlangga has been going well. Radiographers and dentistry students have mostly implemented established SOPs, such as using masks, rinsing films before washing and wearing sterile gloves when washing films. Another factor is the nature of Streptococcus mutans itself. Although Streptococcus mutans is a facultative anaerobic, optimal atmospheric conditions for the growth of these bacteria are anaerobic or only

contain a low percentage of oxygen with 5-10% carbon dioxide (Zhou, & Li, 2015).

Other properties of *Streptococcus mutans* are oral primary colonizer, thus these bacteria need a place to colonize and form biofilms (Rukayadi, Kim, & Hwang, 2008). These bacteria are generally found on the surface of teeth that are isolated and synthesize various extracellular polysaccharides such as sucrose. These polysaccharides help the formation of bacterial colonies (Zhou, & Li, 2015). The conditions in the developer solution also support the growth of *Streptococcus mutans*. The absence of a surface where the *Streptococcus mutans* attaches to the developer's solution is a factor in the non-presence of the *Streptococcus mutans* colony. This happens to the Aquadest solution as a sterile solution that contains no mineral content so it cannot be a place for growth of *Streptococcus mutans* (Göstemeyer, et al. 2018; Jeani, & Andina, 2019).

Study shows that iron and zinc in certain concentrations can influence the growth of *Streptococcus mutans*, i.e. iron can increase its growth by 2.8 times, while zinc can increase growth doubled (Aranha, et al. 1982). Iron can also increase the aggregation ability of *Streptococcus mutans* and the development of biofilms in the fluid phase or in the adherent phase (Francesca, et al. 2004). Besides that, other studies indicate that the manganese element at a concentration of 50-100  $\mu\text{M}$  shows the maximum growth of *Streptococcus mutans* (Arirachakaran, et al. 2007). In this study, the developer solution condition does not

support the growth of *Streptococcus mutans* bacteria, so there is probably no influence from these elements. In addition, the levels of iron, zinc and manganese in PDAM water are not counted. It is possible that the levels of iron and manganese in PDAM water are too low or too high to affect the growth of *Streptococcus mutans* bacteria. The optimal iron concentration is around 3.6  $\mu\text{M}$  and if it reaches 5.4  $\mu\text{M}$  it will reduce the stimulation of *Streptococcus mutans* growth. While in the zinc element, the optimal level is 0.4  $\mu\text{M}$  and when it reaches 0.8  $\mu\text{M}$  will reduce the growth stimulation of *Streptococcus mutans* as well (Aranha, et al. 1982). The manganese element will withstand the growth of several strains of the bacterium *Streptococcus mutans* when its concentration reaches 200  $\mu\text{M}$  (Arirachakaran, et al. 2007). The levels of the element iron, zinc, and manganese may have reached these maximum levels and actually reduce the stimulation of *Streptococcus mutans* growth.

## CONCLUSION

The high growth of *Streptococcus mutans* bacteria in the developer solution with a water supply solvent can be due to differences in the content of the two solvents. In the aquadest solvent developer solution, no surface was found that could be a place for attachment of *Streptococcus mutans* bacteria in the developer solution. Hence, the aquadest solvent developer solution is more effective to minimize the growth of *Streptococcus mutans* bacteria.

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