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ORIGINAL ARTICLE

Evaluation of Virucidal Activity of Reused Gluteraldehyde Solutions

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ABSTRACT

Background*: Heat sensitive medical devices are decontaminated between patients in 2% alkaline gluteraldehyde (GTA) baths. Baths are often reused for 14 days. Many busy endoscopy or surgery units also disinfect such instruments with contact times less than the recommended by the manufacturer.* ***Aim of the work****: is to evaluate the virucidal activity of alkaline glutaraldehyde (GTA) against both RNA and DNA virus models at different contact periods regarding reusing durations as well as to assess its concentration, protein contents and the solution pH.* ***Methodology****: We collected daily samples of disinfectant from a manual bath at a local hospital over the 14-days of the reuse cycle. Control samples were also collected from a similar bath of glutaraldehyde but did not receive any instruments. The virucidal activity of alkaline gluteraldehyde was evaluated after 5, 15 and 20 minutes contact time, using Rift Valley Fever virus (RVFv), Herpes simplex virus type I (HSV- I), Adenovirus- 40 (Adv- 40), and Poilovirus type 3 (Poliov-3). The criterion of efficacy was a minimum of a 3-log^{10}-unit reduction in the infectivity titers of the virus tested. Solution concentration, protein contents and pH were estimated for each sample.* ***Results****: Shorter exposure time of 5 and 15 minutes were critical and not efficient when pH and concentration decrease and protein load increase. An exposure time of 20 minutes improve the efficiency of GTA regarding (HSV-1) and (RVFv), however showed failure to eradicate polioviruses on day 10 (2.55 log reduction) and adenovirus on day 12 (2.74 log reduction) of the reuse cycle. An inverse relation between protein accumulation and gluteraldehyde concentration were observed. These finding emphasize the importance to monitor the disinfectant especially with increasing the threat of AIDS, hepatitis viruses and the resurgence of tuberculosis.*

INTRODUCTION

Glutaraldehyde (GTA) is most commonly used high-level disinfectant for medical equipment such as endoscopes[1]. Heat-sensitive medical devices, need decontamination by chemicals disinfectants between cases. With the increasing number of endoscopy operations[2] and the rising risk of infections caused by the human immunodeficiency virus, hepatitis viruses and mycobacteria [3,4], chemical disinfectants must be monitored cautiously[5].

Glutaraldehyde does not harm lensed devices or rubber, and is noncorrosive to metal. Glutaraldehyde is not suitable for cleaning noncritical surfaces as it is highly toxic as well as expensive[6].

The commercial names of glutaraldehyde-based disinfectants include, but are not limited to, Cidex, Sonacide, Sporicidin, Hospex, and Omnicide[7]. Glutaraldehyde aqueous solutions are acidic and in this condition are not sporicidal. The solution is “activated” (made alkaline) by use of alkalinating agents to pH 8.0–8.5. The shelflife of activated solution is 14 days[6].

During the 14-days reuse cycle, the microbicidal activity of a chemical disinfectant can be influenced by different factors. Dilution[4], exposure or contact time, pH level, organic load, cumulative number of instruments immersed in the bath, aging, and the temperature at which alkaline glutaraldehyde is used are critical factors for the germicidal potency[8].

Mode of Action or the biocidal activity of glutaraldehyde results from its alkylation of sulfhydryl, hydroxyl, carboxyl, and amino groups of microorganisms, which alters RNA, DNA, and protein synthesis or it may produce cross-links of the 'correct' molecular length and interact with the sequence of proteins on the outer coat of viruses [9].

Aim of the Work:

The aim of the present work is to evaluate the virucidal potential of alkaline glutaraldehyde (Cidex) against different viruses; both RNA and DNA models regarding reusing durations, and to assess its concentration, protein contents and the solution pH.

MATERIAL AND METHODS

Cells:

Vero cells were kindly supplied from R&D sector VACSERA–Egypt, it was maintained according to the manufacturer protocol, where growth medium (MEM-E) Minimum Essential Medium supplemented

with Earle's salt (EMEM) and 10 % Fetal calf serum and antibiotic (1mg streptomycin and 100 IU penicillin) was decanted and cells were treated with 5-10 ml of 0.25% trypsin solution. Fetal calf serum, trypsin and E–MEM were supplied from (GIBCO-USA), Vero cells were splitted according to the time of need. Cell growth was examined using inverted microscope (Hund –Germany)

Viruses:

Virus models: The following virus models were kindly supplied from Prof. Dr. Aly Fahmy Mohamed Head of R& D Sector VACSERA:

- Rift Valley Fever virus Menya-sheep-258 [M/S/258] (RVFv): The used viral stock solution was $10^{5.66}$ $TCID_{50}$.
- Adenovirus serotype 40 (Adv-40): The used viral stock solution was $10^{4.75}$ $TCID_{50}$.
- Herpes simplex virus type I (HSv- I). The used viral stock solution was $10^{4.5}$ $TCID_{50}$.
- Poliovirus serotype 3 (Poliov): The used viral stock solution was $10^{6.6}$ $TCID_{50}$.

Glutaraldehyde Solution, baths and sampling:

A product containing about 2% glutaraldehyde, was used in an Endoscopy Unit, at local hospital in Zagazig city. The activator provided with the product was added to it just prior to filling the disinfectant baths. The directions for use is 15-20 minutes exposure to activated disinfection solution at 20°C to destroy vegetative organisms including *P. aeruginosa*, pathogenic fungi, and enveloped as well as nonenveloped viruses[10]. The used instruments are prewashed in warm tap water containing an enzymatic detergent, rinsed in fresh warm tap water, and then exposed to the disinfectant without drying. The disinfectant was reused for 14 days. The activity and validity of solution were tested according to manufacturer instructions using chemical strips which are provided with the product. The solution in the manual bath was first thoroughly mixed before withdrawing the samples. Approximately 25 ml of the disinfectant was collected daily from the bath.

Evaluation of virucidal activity of the gluteraldehyde samples:

Hundred µL of test virus models were mixed with 900 µL of disinfection solution in sterile tubes. The action of the product was stopped using Phosphate buffer saline (PBS) to reduce its cytotoxicity, after a given period of contact (5, 15 and 20 min) [10,11]. Virus disinfectant mixture were 10 fold serially diluted from 10^{-1} to 10^{-8} in E- MEM. Prepared dilutions were dispensed onto 24 hrs precultured Vero cell line in 96 well plates (TPP-Swiss). Infected cultured plates were incubated in CO_2 incubator (Jouan –France).

Tissue culture plates were daily examined for detection of cytopathic effect (CPE). 50 % end point induced CPE was determined according to Reed and Muench (1938) as follow [12].

50 % endpoint (TCID 50) =

$$\frac{\text{(percentage of CPE at dilution next above 50\%)} - 50\%}{\text{(percentage of CPE next above 50\%)} - \text{(percentage of CPE next below 50\%)}} \times \log \text{dilution.}$$

Estimation of the virucidal activity:

The criteria used for assessment of virucidal activity is a 3 log reduction in virus titre. This is effective if the virus has a high titre, i.e. $>10^8$ ID_{50} mL^{-1} and it is still feasible to show a 3 log reduction in virus titre [13,14].

Measuring of disinfectant pH: The pH of each disinfectant sample was determined using pH meter (Thermo Scientific, USA).

Estimation of glutaraldehyde concentration.

Gluteraldehyde concentrations were measured according to method described in Mbithi and his colleagues study[5]. The protocol was provided from Surgikos Canada, Inc. (Peterborough, Ontario, Canada), and it is depended on the reaction of alkaline glutaraldehyde with hydroxylamine with the production of hydrochloric acid (HCl) as one of the by-products, which is in turn titrated against 0.1 N NaOH.

Measuring of protein accumulation.

Estimations of protein in the disinfectant samples were made using LumaSpec Spectrometer, USA.

Cumulative loading of instruments.

The endoscopy unit was requested to maintain precise record of the number of instruments processed daily through that reuse cycle of the bath.

Controls:

1. **Gluteraldehyde control solution** For a control, a manual bath was set up with 4 liters of freshly prepared and activated, alkaline glutaraldehyde, solution and placed in the same room as the test bath.
2. **Cell control:** 4 rows on each plate was not infected with virus but contained only growth medium
3. **Viruses control :** Non disinfectant treated virus control infected cells was used as positive virus control to compare reduction % of virus infectivity titer

RESULTS

PH levels.

Figure 1 represents the pH levels in control and manual (reuse) baths. At the first day of activation of gluteraldehyde the pH values of control and manual bath samples were 8.5. The pH values of control bath showed no significant change over the reuse period. As regard the reuse bath, pH levels showed early drop during reuse cycle and reached to 7.2 on day 14.

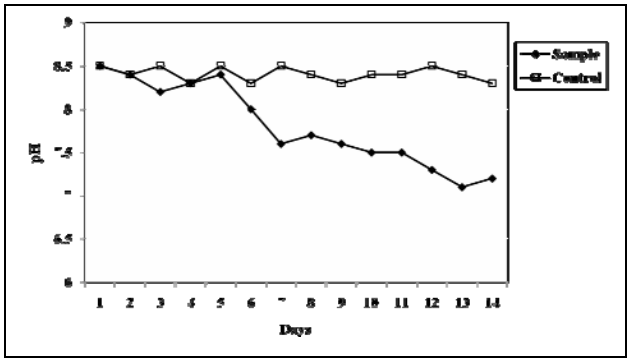

Fig. 1: The pH values of samples from control and reuse bath.

Concentration of gluteraldehyde:

From figure 2 we can see that gluteraldehyde concentration in control bath decreased from 2.25 on day one to 1.75 on day 14. The solution concentratin of samples from manual bath went from 2.25 to 1.1 on last day.

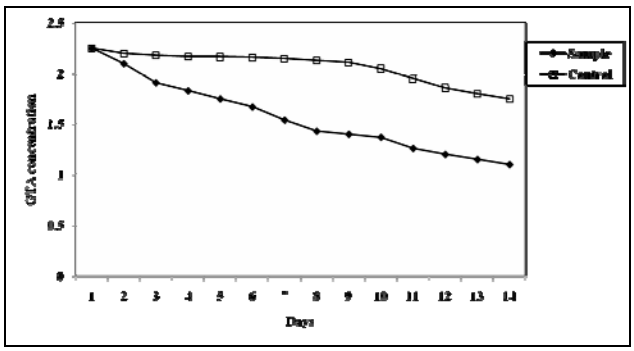

Fig. 2: Concentration of GTA in control and reuse bath during the reuse cycle.

Protein concentration:

Samples from manual bath show gradual rise in protein concentration during the reuse period. The rate of protein accumulation was slow in the first 7 days and becomes higher rates during the remaining period of reuse cycle reaching to 1180ug/ml on day 14. As regard control bath all samples were protein free (Figure 3).

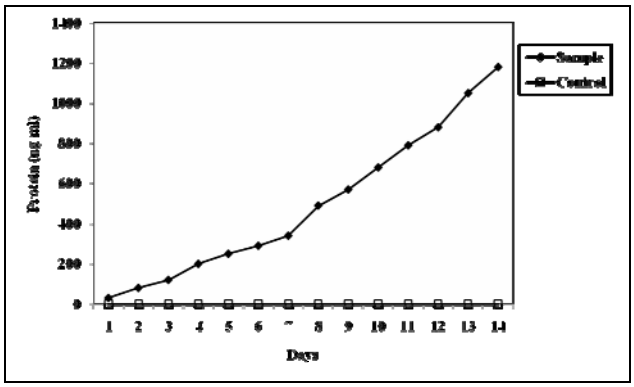

Fig. 3: Protein concentration in control and reuse bath.

From both figures 2 and 3 we can notice an inverse relation between GTA concentration and protein contents, as the concentration decreases the protein contents increases.

Cumulative number of instruments.

Figure 4 shows the number of instruments processed in the manual bath during the 14 days of the reuse cycle.

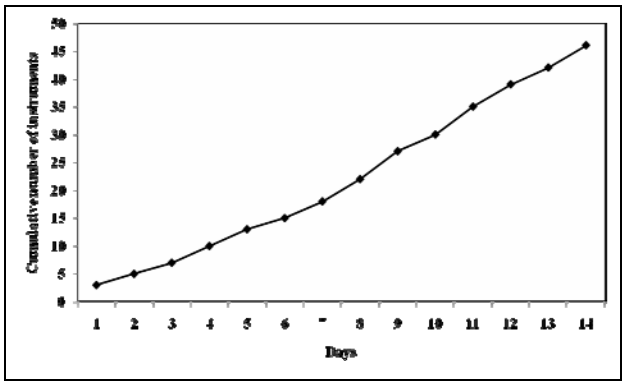

Fig. 4: Cummulative number of instruments immersed in the reuse bath during the 14 days cycle.

Virucidal activity of the Gluteraldehyde at room temperature: (Table1)

- Control samples remained effective and showed virucidal activity against all tested virus models at room temperature during the 14 days at both 15 and 20 min exposure time.
- Samples from manual disinfection bath, when tested with exposure time 5 minutes show 1st failure on 3rd day with polioviruses followed by adenovirus on 5th day of reuse cycle.
- As regard exposure time of 15 minutes, samples showed failure on day 5 for poiliovirus and on day 10 for both Herpes and Adeno viruses' models. On day 10 Herpes virus shows border line log reduction 2.84, however Adenovirus showed lower log reduction of 2.3 rate. As regard RVFv it showed failure with 15 min contact time on day 12 with log reduction of 2.66.
- When extending the contact time to 20 minutes at room temperature, samples showed the efficacy criterion as regard RVFv and herpes viruses; however this didn't improve the performance of the sample against poliovirus or adenoviruses which showed failure on day 10 and 12 of reuse cycle, respectively. At that time the log difference of Poliovirus was 2.55 and of Adenovirus were 2.74.

Table 1: Days of the reuse cycle on which gluteraldehyde gave first failure with different viral models.

Test virus	*Contact time (min)*[a]	*Days of reuse of manual bath*
RVFV	5	6
	15	12
	20	>14[b]
Herpesvirus- I	5	8
	15	10
	20	>14[b]
Adenotype- 40	5	5
	15	10
	20	12
Poliovirus- 3	5	3
	15	5
	20	10

[a] At room temperature.
[b] > 14 efficient for reuse during the whole cycle.

DISCUSSION

Numerous chemical disinfectants are used in the health-care settings. These include alcohols, chlorine and chlorine compounds, hydrogen peroxide, phenolics, formaldehyde, glutaraldehyde, ortho-phthalaldehyde, iodophors, peracetic acid, and quaternary ammonium compounds[6]. Glutaraldehyde is a broad spectrum disinfectant of aldehydes family[16].

There is a growing concern about the probable negative influence of disinfectant dilution, pH changes, and protein accumulation in reuse baths on the virucidal activity of alkaline glutaraldehyde disinfectant [17&18].

Glutaraldehyde was noted to become diluted, and its concentration declined after a few days of ruse cycle. The decrease in the concentration occurs because instruments are not thoroughly dried after washing and water is carried into the disinfectant basin with the instruments, which dilutes its effective concentration [19] as well as increases the solution's volume. At the end of reuse cycle GTA concentration was 1.2%. In another study by Whyman and his colleagues, they found that the levels of the chemical to fall below 1% [20]. The discrepancy between our study and their study may be attributed to two reasons the first that they examined disinfectant samples from automatic bath and the second is to the difference in the number of the instruments disinfected.

This study noticed also decrease of GTA concentration in control bath. This can be explained by the polymerization of solution by aging. As alkaline pH cause polymerization of the glutaraldehyde molecules, this polymerization blocks the active aldehyde groups of the glutaraldehyde molecules which are responsible for its microbicidal activity [6].

This study found that chemical strips, which shows a change of color if the glutaraldehyde solution is still active, failed to detect this drop in the disinfectant concentration. This results agreed with Power and Russell [21].

Alkaline glutaraldehyde is considered to be more resistant to interference by organic matter than most other disinfectants and provide excellent biocidal properties in the presence of organic matter (20% bovine serum); [6]. In our study an inverse relationship between protein accumulation and GTA concentration was observed.

The cumulative number of instruments subjected to the disinfectant under reuse state also contributes to decrease of activity as a result of increase protein load and decrease the solution concentration [22].

In heat sterilization a, highly-resistant organism, e.g. *Bacillus stearothermophilus* is used to test the efficacy of sterilization and *Streptococcus faecalis* is chosen for tests of heat disinfection. It is well known that after spores, viruses have the highest resistance to 2% GTA solutions [23].

An important hypothesis was put forward and modified in 1983 in which it was proposed that viral susceptibility to disinfectants could be based on whether viruses were "lipophilic" in nature, because they possessed a lipid envelope or "hydrophilic" because they did not. Klein and Deforest [24] further classified viruses into three groups, A (lipid containing), B (nonlipid picornaviruses), and C (other nonlipid viruses larger than those in group B, eg. Adenovirus)[16].

In this study four viral models of different categories were investigated, RVFv and Herbes viruses are models for lipid containing virus, whereas poliovirus and the adenovirus are nonlipid containing viruses. Those models are widely used as surrogates for assessment of virucidal efficacy of disinfectants against all pathogenic viruses (25& 26). Also those viruses are characterized by that high titers can be easily achieved and accurate measurements of log reductions can easily be estimated [17].

In this study, viral assay was carried out using the most-probable-number method. This method is more accurate when 96-well culture plates are used in place of tissue culture tubes. This technique is preferred as, it can measure the activity of antiseptics and disinfectants, cell cultures are easily reproducible, and assays can be achieved with a large number of replicates. However, such methods are not valid to non-cultivable viruses [18].

This study found that, control bath was able to eradicate the infectivity of the investigated virus at 10 and 20 min all over the reuse cycle, this come in accordance with Elkholy and his colleagues who declared that GTA under clean condition (without biological load) was effective virucidal at 10 and 20 min contact [18].

This study found that shorter contact times (5 or even 10 min) seem to be adequate to inactivate viruses during the early few (3-5) days of the reuse cycles. These periods of contact would become critical if disinfectants concentrations changes by dilution or pH decreases. Our results agreed with the study of Bailly and his colleagues [17].

In this study, the used virus's models showed different degree of resistant to GTA, coming first poliovirus followed by adenoviruses, however RVFv and herbesviruses are more susceptible to GTA. That finding confirmed those of Tyler and his colleagues [17] who declared that, poliovirus is more resistant to disinfectants than herpesvirus. They explained such differences, to being either lipophilic or hydrophilic viruses.

In this study the reduction of poliovirus log were lower than that in Tyler and his collegues study. They showed a log reduction greater than 2.6 at 5 and 15 min, in or study the log reduction didn't exceed 1.85 in all sample at similar contact time [17]. The difference between our study and their study can be explained by the procedures followed in their study. Tyler and his colleagues used a high titer of virus, suspended in an organic medium. The virus suspension was dried on a cover slip, and then exposed to the chemical disinfectant. The disinfectant was then removed by rinsing before assaying the virus. Such procedure has the limitation of probable loss of virus during rinsing step thus their study recorded higher reduction in poliovirus than ours.

Capsid proteins are predominantly protein in nature, glutaraldehyde is known as protein cross-linking reagent that reacts strongly with amino or sulfhydryl groups which would interfere with adsorption of virus to the host cell or with uncoating. This might also possess virucidal activity if destruction of viral capsid lead to the release of a potentially infectious nucleic acid and that viral inactivation would be complete if the viral nucleic acid is also destroyed by the disinfectant [27].

Mechanisms of viral resistance to disinfectants include, multiplicity reactivation which was supported by of Young and Sharp [28], viral aggregation, and the possibility of viral adaptation to new environmental conditions [16].

As a conclusion, gluteraldehyde disinfectant should be monitored precisely for pH, concentration and protein load during the reuse cycle, especially with increasing threat of AIDS and tuberculosis. This study recommends increase the contact time (>20 min) or increase the temperature of the GTA bath especially near the last days of the reuse cycle. Further studies are recommended to determine the precise mode of action of of GTA on viral capsids and nucleic acid. This study also recommend testing virucidal of gluteraldehyde on non cultivable viruses especially the hepatitis C virus (HCV) and the human immunodeficiency virus (HIV) by quantitative PCR and the human hepatitis B virus (HBV) by detection of HBs Ag using quantitative ELISA.

REFERENCES

1. Cheung RJ, Ortiz D, DiMarino AJ: GIT endoscopic reprocessing practices in the United State Gastrointest Endosc 1999; 50:362-8.
2. Axon ATR: Disinfection of endoscopic equipment. Bailiere's Clin Gastroenterol 1991; 5:61-77.
3. Alvarado CJ, Stolz SM, and Maki DG: Nosocomial infection and pseudo infection from contaminated endoscopes and bronchoscopes-Wisconsin and Missouri. Morbid. Mortal Weekly Rep 1991; 40:675-677.
4. Hanson PJV, Clarke JR, Nicholson G, Gazzard B, Gaya, D Gor, Chadwick M V, Shah N, Jeffries DJ, and Collins JV: Contamination of endoscopes used in AIDS patients. Lancet, 1989; ii:86-88.
5. Mbithi, J N, Springthorpe V S, Sattar S A, and Pacquette M: Bactericidal, virucidal, and mycobactericidal activities of reused alkaline gluteraldehyde in an endoscopy unit. J. Clin. Microbiol 1993; 31:2988–2995.
6. Rutala WA, Weber DJ, and the Healthcare Infection Control Practices Advisory Committee (HICPAC)- CDC 2008.
7. National institute of occupational safety and health, publication, 2001
8. Gelinas P, and Goulet J: Neutralization of the activity of eight disinfectants by organic matter. J. Appl. Bacteriol., 1983; 54:243-247.
9. Scott EM & Gorman SP.: Glutaraldehyde. In: Block SS, ed. Disinfection, sterilization, and preservation Philadelphia: Lippincott Williams & Wilkins, 2001; 361-81.
10. Eubanks RD and Farrah SR: Inactivation of poliovirus and other enteroviruses by solutions of sodium fluoride at low pH. Applied & Environmental microbiology: 1981; 686-689.
11. Tyler R, Ayliffe GAJ and Bradley C: Virucidal activity of disinfectants: studies with the poliovirus Journal of Hospital Infection 1990; 15, 339-345.
12. Reed LT and Muench HA: Simple method of estimating fifty percent end points. The American Journal of Hygiene 1938; 27(3): 493-498.
13. Bellamy KA: Review of the test methods used to establish virucidal activity.J Hosp Infect 1995; 30(Suppl.):389—396.
14. Canadian General Standards Board. Assessment of efficacy of antimicrobial agents for use on environmental surfaces and medical devices. CAN/C.G.S.B.-2.161-M91. Canadian General Standards Board, Ottawa, Ontario, Canada, 1991.
15. McDonnell G, Russell AD: Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev 1999;12:147-79.

16. Bailly JL,Chambron M, Peigue-Lafeuille H, Laveran H, Champs C de and Beytout D: Activity of glutaraldehyde at low concentrations (0.2 %) against poliovirus and its relevance to gastrointestinal endoscope disinfection procedures. Appl Environ Microbiol 1991; 57:1156–1160.
17. Elkholy YS, Hegab AS, Ismail DK &Hassan RM: Evaluation of a novel commercial quaternary ammonium compound for eradication of Mycobacteria, HCV and HBV in Egypt Journal of Microbiology 2016; 54 (1): 39–43.
18. Kleier DJ, Averbach RE.: Glutaraldehyde nonbiologic monitors. Infect Control Hosp Epidemiol 1990;11:439-41.
19. Whyman CA, McDonald SA and Zoutman D: Unsuspected dilution of glutaraldehyde in an automatic washer for flexible fibreoptic endoscopes. Can. J. Infect. Control, 1991; 6:91-93.
20. Power EGM and Russell AD: Assessment of 'Cold Sterilog Glutaraldehyde Monitor.' J Hosp Infect 1988; 11:376-380.
21. Leong D, Dorsey C, and Klapp M: Dilution of glutaraldehyde by automatic endoscope machine washers: the need for a quality control program. Am J Infect Control 1991; 19:86.23
22. Borick PM, Dondershine FH, and Chandler VL: Alkalinized glutaraldehyde, a new antimicrobial agent. J Pharm Sci 1964; 53:1273-1275.
23. Klein M and Deforest A: Principles of viral inactivation, p. 422–434. InS. S. Block (ed.), Disinfection, sterilization and preservation, 3rd ed. Lea & Febiger, Philadelphia, Pa., 1983
24. Sauerbrei A, Sehr K, Eichhorn U, Reimer K & Wutzler P: Inactivation of human adenovirus genome by different groups of disinfectants. Journal of Hospital Infection 2004; 57, 67–72.
25. Tuladhar E, Terpstra P, Koopmans M, Duizer E: Virucidal efficacy of hydrogen peroxide vapour disinfection. Journal of Hospital Infection, 2012; 80 110e115
26. Abed El Gaied HA, Hashem AE, El Tayeb O and Mohamed AF: Evaluation of inactivation efficacy of Sabin Polio Virususing different inactivating agents and related immunogenicity. International Journal of Microbiological Research 2010; 1(3):114- 122.
27. Young D C and Sharp DC: Virion conformational forms and the complex inactivation kinetics of echovirus by chlorine in water. Appl Environ Microbiol 1985; 49:359–364.