MICROBICIDE LUBRICATING GEL
FOR ADDED PROTECTION
AGAINST INFECTION
INDUCED BY STD’s
DURING SEXUAL RELATIONS
**Index**

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1. Background

During the last decade the rapid spread of STD's and the attendant fear of its terrifying consequences for mankind has created a sense of urgency for exploring both short and long-term solutions for this modern plague.

One of the most common forms of transmission of STD’s are via human sexual practices and habits. During sexual intercourse, there are primarily three means of transference of STD’s from one person to another. First, is the result of the friction caused by the penis during penetration, which tears small and sometimes invisible blood vessels (capillary vessels) of both sexual partners. The blood is consequently commingled and the viruses are transferred from one partner to the other. The second method is transference of the STD's via the different lubricants which the body secretes during sexual relations. It has been found, e.g. that the HIV can reside in the semen or even reside, on or in the spermatozoa. Thus, the third method is via the semen which, during the sexual act, may carry STD’s and transfer them to the partner.

The most common way of avoiding infection by the HIV during sexual relations is by the use of a condom, which is placed on the penis prior to copulation. The condom prevents direct contact between the two sexual members or their body fluids, and holds the sperm. As far as it is known, the HIV can not penetrate the rubber material of which the condom is made but if the condom is torn or damaged, then transmission may occur. Condoms are considered to give less than 85% safety for preventing transmission of diseases (6,7).

There are many people who do not like using a condom during sexual relations, mainly because of the odd feeling, or the indirect contact which may interfere with or diminish sexual satisfaction.

In a survey (46) conducted on 600 students with an average age of 23 years in the Tel Aviv University, less than one out of four, (24.3%) were using condom. 26.7% of the males and 11.9% of the females, had sexual relations with more than 10 partners during the past three years. Similar results were obtained in the University of Toronto, Canada, and in a Research conducted on 1,000 patients visiting a clinic in West End London. (5,7)

Gel-x™ is intended as a partial protection for people who do not use, or resist the notion of using a condom, or are desirous of adding another safety factor, in addition to using a condom. Gel-x™ provides a nearly safe procedure, which will help to reduce transference of the HIV from one's sexual partner, thereby reducing both risk and anxiety.

Gel-x™ is an antiseptic lubricant to be used as a prophylactic device for reducing the risk of infection by the human immunodeficiency virus, (herein: "HIV"), and other viruses like Herpes Simplex Virus (HSV) or Cytomegalovirus (CMV), Hepatitis B and C, or bacteria like Syphilis or Gonorrhea, or parasite like Chlamydia or Trichomoniasis, all of them are transferred during sexual relations, (herein: "STD’s").

Gel-x™ lubricant also facilitates sexual intercourse in cases of vaginal dryness or atrophy, and prevents over-friction and damage to the tissues.

The purpose of Gel-x™ is to destroy the HIV, Herpes Simplex Virus, Cytomegalovirus, Hepatitis B, in the body fluids, which might transfer the virus to ones sexual partner during copulation. This can be achieved by interposing the product between the two sexual members,
and in the body liquids as well, so that it will destroy the HIV before it enters into the inner tissues of one's sexual partner. It is obvious, therefore, that Gel-x™ must be in, and on, the sexual members, which are participating in the sexual act, and thus will be able to instantly destroy the virus which might be transferred otherwise.

Gel-x™ lubricant will reduce damage to blood vessels during the sexual act, and will increase the enjoyment of the participants. Its antiseptic phenomenon will destroy the HIV which is presumed to be in the body fluids, as it will be in contact with the body fluids present during the sexual act. In addition, the antiseptic will inactivate a high percentage of the spermtocytes and will prevent them from proceeding into the uterus.

In order to use Gel-x™ before, or during, the sexual act, the container of Gel-x™ provides a method of application that is easy to use and does not disturb the sexual act, and still retains the effectiveness of the lubricant.

Gel-x™ provides options for both Male and Female.

For the Male, it is packed in a specially designed single use, disposable, sterile sealed bag which turns into a sachet when peeled apart, by insertion of the erect organ into the sachet the organ becomes lubricated.

In the female controlled version, the GEL-X is injected into the vagina by means of an applicator. The gel, being non-liquid, remains in the vagina during and after copulation.

In both versions, GEL-X is water soluble, and can therefore be washed away by water after the sexual act.

2. Acquired Immunodeficiency Syndrome (AIDS)
3. And Human Immunodeficiency Virus (HIV)

Acquired Immunodeficiency Syndrome (AIDS), a lethal and incurable disease, is caused by a RNA retrovirus, Human Immunodeficiency Virus (HIV). The disease is manifested by suppression of the immune system.

The mean incubation period from infection by HIV and seroconversion to onset of AIDS was estimated to be 2-5 years (1), but lately this estimate was revised and the incubation period is believed now to be about 8-10 years. This means that a long period of infectiousness is possible (2).

Furthermore, it is estimated that from every case of AIDS, 50 to 100 people may be infected. Extrapolations based on these estimates suggested that about 1,000,000 people were already infected at the time of the study (1987) (1). Thus AIDS has moved from the stage of mere disease to the stage of a plague.
4. HIV, AIDS and Sexual Relations

HIV can be found not only in the blood but also in other body fluids. Thus O'Shea et. al\(^3\) tested the blood, semen, vaginal secretions, saliva and cervical fluid in 53 HIV seropositive patients (46 men and 7 women) using enzyme immunoassay (EIA). Of these, HIV was isolated in the blood of 38 patients (71.4%). HIV was also found in the semen of 11% of the patients (2/18) and in the genital secretions of 1 woman out of 6 (16.6%). No HIV was isolated from the saliva and cervical fluid.

The low incidence of HIV in semen and genital secretions might reflect insensitivity of the assay method as in another study, cited by Alexander in her review\(^2\), more HIV were isolated in the semen than in the blood when using the more sensitive method of gene amplification by means of polymerase chain reaction.

HIV can be found also in or on the sperm as was reported by Bacceti et. al.\(^4\). They detected HIV adhering to motile spermatozoa ejaculated by 5 AIDS patients as well as in the cytoplasm of the spermatozoa.

Various studies cited in Alexander's review\(^2\) report detection of high rates of HIV seropositivity in the presence of sexually transmitted diseases (STD) like Herpes Simplex (HSV) or Cytomegalovirus (CMV), implicating STD as a risk factor in HIV infection.

As HIV is present both in blood as well as in the semen, sperm and female genital secretions, it is clear that a major common mechanism of HIV infection and spread is through transmission during the sexual act.

Runganga AO, Kasule J\(^75\) state that heterosexual intercourse accounts for 80% of HIV transmission in Sub-Saharan Africa.

5. Protection against HIV Transmission

From the foregoing discussion it is clear that a complete barrier used during sexual intercourse would prevent the transmission of HIV. Such a complete barrier is the use of a condom recommended by the U.S. General Surgeon.

However, use of condoms is not so widespread, even after a national information campaign, as was evidenced by a survey\(^5\) conducted in Britain during 1987 on women attending a genitourinary clinic. Of the 1066 women surveyed, 672 (63%) never used condoms during sexual relations with their regular partners. A more alarming finding was that out of 424 women, 225 (53%) never used condoms when having sexual relations with non-regular partners.

In another study\(^7\), the percentage of heterosexual males using condoms was only 27.5% and that of heterosexual females was 25.8%.

Moreover, given the 10% to 15% contraceptive failure rate of condoms, the undetermined but significant slippage and breakage rate\(^6,7\), the condom cannot be viewed as a completely safe means for preventing sexually transmitted diseases and a virucidal back up chemical barrier is apparently needed.
Such a chemical barrier should possess, besides its virucidal activity, the following performance characteristics: low human toxicity, compatibility with latex and reliable spermicidal and broad-spectrum microbicidal activity (7).

6. Chlorhexidine - STD's and other Microbicidal Activity

Chlorhexidine, a bisbiguanide that exists as a bication in aqueous solution (8), is a well-known antimicrobial agent with a broad-spectrum activity. It is effective against a wide variety of bacteria including Gram positive, Gram negatives, aerobes and anaerobes (9).

Chlorhexidine achieves its antibacterial effect both by disruption of the permeability barrier of the cells and also by blocking electron transfer in the cytochrom system. Both of these effects can be attributed to combination of chlorhexidine with the cytoplasmic membrane, causing an alteration or breakdown of its structure (10).

From In-Vivo and In-Vitro studies which have been conducted (56, 57, and 58), we learn that CHG in concentrations between 0.05% to 0.02% can destroy the following STD's: -Chlamydia, Gonorrhea, Syphilis, and Trichomoniasis.

Modak S, et. al. (69) confirms that CHG gloves rapidly inactivate infectious pathogens (e.g., bacteria, fungi, parasites, retrovirus, hepatitis B virus, as well as to lymphocytes and macrophages).

7. Uses of Chlorhexidine

Since its introduction in the 1950's, chlorhexidine is one of the most widely used antimicrobial agents because of its safety and efficacy and has been found to have widespread applications in medicine as an antiseptic for disinfection of skin, wounds, mucus and membranes in various preparations based on its gluconate, digluconate, acetate and diacetate salts (21).

It is listed in the British Pharmacopoeia both as a 20% aqueous solution (8) as well as a Chlorhexidine Cream (11) and Chlorhexidine Dusting Powder (12).

It is listed in the U.S. Pharmacopoeia both as a 20% aqueous solution of Chlorhexidine Gluconate and Chlorhexidine Gluconate Oral Rinse.

It has been used for hand washing and preoperative disinfection for at least three decades in a huge number of hospital (22). Rushton (23) reported in 1977 that over 20 products containing chlorhexidine and its salts were marketed.

Osmundsen (24) notes that in Denmark, chlorhexidine gluconate and acetate are used extensively in creams, emulsions (Hibiscrub), aqueous and alcoholic solutions (Hibitane, Savlon) and in impregnated gauze (Bactigras). He also notes that during the last few years chlorhexidine has become a popular remedy for treatment of skin infections, wounds and ulcers in Denmark.

8. Oral Uses of Chlorhexidine
Chlorhexidine has been found to be an effective anti-plaque agent (45). Clinical studies have shown that 0.2% oral chlorhexidine rinse or 2% chlorhexidine topical gel effectively inhibits the accumulation of supragingival plaque (13).

Robinson (83) concludes that irrigation with aqueous chlorhexidine solution is almost diagnostic of the condition of gingivitis and oral cleaning.

It is also used in the initial phase of acute narcotizing ulcerative gingivitis and in the postoperative period following periodontal and oral surgery (25).

9. Chlorhexidine as a Wound Cleanser

The effect of chlorhexidine on wound healing compared to saline was investigated in an in-vivo study (26) on 45 rats in which skin wounds were artificially inflicted. An aqueous gel containing 0.05% chlorhexidine was applied to the wounds for up to 7 days. The results showed that there were no differences between chlorhexidine and saline in their effect on collagen formation in the granulation tissue as well as no differences in deoxyribonucleic acid concentrations and histological examinations.

As new microsurgical techniques for repairing small vessels require asepsis and irrigation, a study (27) was conducted with the aim of comparing the in-vivo toxicity of 0.05% chlorhexidine vs. saline and 10% povidone-iodine in open wound provoked in rats by microsurgical techniques. In the 30 rats treated with chlorhexidine, 2 rats had thrombus compared to 2/30 treated with saline and 27/30 treated with Povidone-iodine.

In both of these studies, the authors conclude that a 0.05% chlorhexidine solution applied to open wounds is virtually innocuous.

10. Chlorhexidine Applied to the Penis

As patients with spinal cord injuries who are undergoing catheterization are prone to urine tract infections, three studies were conducted with the aim of assessing the effect of chlorhexidine on development of urine tract infections.

In the first study (28), patients, who were undergoing intermittent catheterization on several occasions each day, were whole body washed daily with a 4% chlorhexidine gluconate solution with a liberal application of chlorhexidine cream on the glans penis after each catheterization for an 8 week period. The results showed a significant reduction in the bacteriuria without any side effects.

The results of the second study (29), which tested the effectiveness of 0.033% chlorhexidine solution applied to the glans penis in reducing the occurrence of urinary infections in females and bacterial prostatitis in male patients, were inconclusive and the authors conclude that chlorhexidine is not a more effective antiseptic agent than soap. However, no side effects were reported.

The third study (30) compared the effectiveness of a 4% solution of chlorhexidine gluconate against other agents for cleansing the genital area and reducing the microflora in that region.
A moderate reduction in the populations of P. aeruginosa, K. pneumonia and total aerobes was found, again without noticeable side effects.

11. Chlorhexidine In the Vagina

Vorherr et.al \(^{(31)}\) conducted a study with the purpose of determining the effect of a 4% solution of chlorhexidine gluconate on the microflora in the vagina. 150 women participated in this study were swabbed twice with a sponge soaked with the chlorhexidine solution. Samples taken from the vaginal wall 5 minutes after swabbing showed a 3.5 mean log reduction in the vaginal wall bacterial count.

Group B streptococci (GBS) found in the vagina are transmitted vertically to infants during delivery, and are one of the main causes for morbidity and mortality of neonates, hence the importance attached to preventing transmission of GBS from mothers to infants and the numerous studies dealing with prophylaxis and antisepsis of the vagina during labor.

Sanderson and Haji \(^{(32)}\) investigated the effect of whole body washing with 4% chlorhexidine gluconate solution during the last weeks of pregnancy on the transmission of GBS, but as the washing habits of the 280 women who participated in this study were diverse, no conclusive results were received. It must be noted that no side effects were reported.

The effectiveness of chlorhexidine acetate against 43 strains of GBS, commonly found in the vagina of GBS carriers, as well as against other micro-organisms (S. faecalis, S. aureus, S. epidermidis) was studied \(^{(33)}\). The minimum inhibition concentrations (MIC) were found to be 2 to 32 mg/l. The minimum bactericidal concentrations (MBC) were 1.25-5 mg/l. No growth was exhibited after one-hour exposure (the minimum exposure time) to 0.5 g/l chlorhexidine acetate. The authors conclude that GBS is highly susceptible to chlorhexidine.

Christensen et. al. \(^{(34)}\) conducted a study on the effect of washing the vagina with 0.2% chlorhexidine acetate solution on the incidence of GBS in newborn infants. The vaginas of 18 women were swabbed with the chlorhexidine solution every sixth hour during labour until delivery, irrespective of the membrane being ruptured or not. A marked reduction in GBS colonization in infants without side effects was found.

Chlorhexidine has a long lasting microbicidal activity in the vagina as was demonstrated in the following two studies:

Christensen et. al. \(^{(35)}\) applied a single washing of 0.2% solution of chlorhexidine acetate to the vagina of 31 women, who were persistent carriers of GBS, during delivery. Microbiological tests performed four days after the application showed that 32% of the women were still not recolonized with GBS compared to 17% not recolonized in the control group. The authors conclude that a single vaginal washing with chlorhexidine during delivery suppressed the recovery of GBS from the urogenital tract for at least 4 days.

In the second study \(^{(36,37)}\), chlorhexidine cream was applied to the vagina of 15 pregnant women who were carriers of GBS. Tests performed one hour after application showed a mean log reduction of 1.24 in total aerobic count and 2.72 reduction in total anaerobic count. The activity of chlorhexidine continued even after six hours, reducing the mean log total count of the aerobes and anaerobes by 1.94 and 3.94 respectively.
Burman et al (47) in their study on 4559 women, out of which 2238 women were treated every 6 hours between 2 to 6 consecutive times, with 60 ml CH 2g/l (0.2%), which is equivalent to 120 mg compared to maximum 60 mg in Gel-x™ (12 ml of 5 mg/ml). Out of 2238 women, only 2 reported slight vaginal stinging after two flushings, and 1 reported local irritation for two hours after five CH flushings. No other side effects were reported.

Chantler (48) who used 5 ml gel containing 72 mg CHG (formulated as a 1% gel), and the Editorial Essay of The Lancet (49), concluded that after work which had been carried out for several years at the Manchester University, the agent has spermicidal properties but is not a surfactant and is positively charged. They added that it is compatible with cervical mucus, and it does not seem to disrupt the vaginal epithelium.

Shubair et al (50) conducted their research on the effect of Chlorhexidine Gluconate douche on normal vaginal flora on 20 Volunteers who were treated daily with 180 ml solution containing 0.5% Chlorhexidine Gluconate (equal to 90 mg per day) for 7 consecutive days. No side effects were observed, even after 30 days. No cases of vaginitis, either yeast vaginitis or bacterial infection were definitely diagnosed as arising as a consequence of the use of Chlorhexidine Gluconate. No untoward effects on the participants were observed.

O'Connor TJ, et. al. (70) tested the effect of low pH, normally present in the female genital tract, and the virucidal activity of CHG (in vitro). The seminal fluid was found to have significant activity against HIV-1 and a selectivity index of approximately 50 compared to less than 5.2 in regular Semen and cervical secretions.

Biggar RJ, et. al. (72), of the Viral Epidemiology Branch, National Cancer Institute, Bethesda, Maryland, U.S.A, examined the efficacy of birth canal washing against prenatal transmission of 3637 women. The intervention consisted of washing the birth canal with 0.25% chlorhexidine, at the time of admission in labour and thereafter, every 4 hours until delivery. No adverse reactions to the intervention procedure were seen.

Taha TE, et. al. (73), of John Hopkins University, Baltimore MD, USA, performed a clinical in vivo trial on 3635 women giving birth over a six month period. Intervention was performed after two months of no intervention, and then followed by three months of intervention and finally, one month of no intervention. The intervention was a manual wipe of the maternal birth canal with 0.25% chlorhexidine solution. Hitherto, there were no adverse reactions related to the intervention among the mothers or their children. In this research, opposed to Biggar RJ, et. al. (72), the conclusions were: Cleansing the birth canal with chlorhexidine reduced early neonatal and maternal postpartum infections problems. The safety, simplicity and low cost of the procedure suggest that it should be considered as standard care to lower infant and maternal morbidity and mortality.
Table I: Partial List of Products containing 1-4% CHG commonly used in the vagina:

<table>
<thead>
<tr>
<th>Product Manufactured By:</th>
<th>Country</th>
<th>Type</th>
<th>% CHG</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLUREXID RHONE POULENC THERAPLIX HIBITANE ICI</td>
<td>France</td>
<td>Foam</td>
<td>1.5%</td>
<td>Vaginal Lubricant &amp; disinfectant</td>
</tr>
<tr>
<td></td>
<td>Britain</td>
<td>Cream</td>
<td>1.0%</td>
<td>Vaginal lubricant &amp; disinfectant obstetric cream</td>
</tr>
<tr>
<td>EKUBA UNIONE CHIMICA MEDICAMENTI BAXIDINE BERGAMON</td>
<td>Italy</td>
<td>Wash</td>
<td>2.0%</td>
<td>For hygienic vaginal washing</td>
</tr>
<tr>
<td>SAVLON ICI</td>
<td>Italy</td>
<td>Foam</td>
<td>1.5%</td>
<td>Vaginal disinfectant</td>
</tr>
<tr>
<td>KLORHXIDN ISOTOP DAK</td>
<td>Denmark</td>
<td>Cream</td>
<td>1.5%</td>
<td>Vaginal disinfectant</td>
</tr>
<tr>
<td>CHLORHEX DELTA WEST OBSTETRIC LOTION</td>
<td>Australia</td>
<td>Lotion</td>
<td>1.0%</td>
<td>Vaginal disinfectant before childbirth and lubricant for midwives &amp; physicians</td>
</tr>
<tr>
<td>HIBISCRUB ICI</td>
<td>Italy</td>
<td>Foam</td>
<td>4.0%</td>
<td>Vaginal disinfectant</td>
</tr>
</tbody>
</table>

12. Absorption of Chlorhexidine

As povidone iodine, which is widely used to treat vaginitis, is absorbed readily into the blood stream presenting a hazard in respect of thyroid function, the absorption of chlorhexidine, an alternative treatment, was investigated.

One study (31) tested the chlorhexidine content in the blood of 30 women before washing their vagina with chlorhexidine and 60 minutes afterwards. **No chlorhexidine was detected in the samples** using a gas chromatograph with 100-ng/ml sensitivity.

In a later study (38), the blood level of chlorhexidine in 196 patients who underwent vaginal washing with 0.2% solution of chlorhexidine acetate during labour was determined. Determination was performed by gas chromatograph with a lower detection limit of 10 ng/ml. Chlorhexidine levels of 10-83 ng/ml were found after three hours, but a second washing done six hours after the first one had not shown any increase in blood levels. The authors conclude that a certain but very limited absorption of chlorhexidine takes place during delivery but is not cumulative.

Shubair et al (50) detected also small negligible amounts of CHG in the serum, between 0.03 g/ml to 0.16 g/ml. Blood sample was taken from each subject within 24 h of final douche application.

Researches conducted on burn patients, e.g. Brougham et al (51) in his research "To Monitor the CH levels in burn patients treated topically" have proved that the CHG which was absorbed in the blood was completely discharged in the urine. **Conclusions show that CHG is not accumulated in the blood, and is discharged through the urine.**
Furthermore, another study (52) concludes that residues of CHG of higher than $10^{-4}$ caused problems, but on ranges from $10^{-4}$ and lower, there were no effects on blood cells.

The highest residues found in Nilsson et al (25) were $83 \times 10^{-9}$ which is a safety factor of over $10^4$ higher than what was determined as not affecting blood cells.

Severyns et al (27) concluded in an in vivo research that CHG at 0.05%, 0.02% and 0.001% was found to have a very low toxicity which was exactly comparable to physiological saline. These results show that an amount of $10^6$ higher than the quantity of residue found in Nilsson et al (25), did not influence revascularization and reacted in the same manner as physiological saline.

The amount of CHG in 10 ml Gel-x™ is 0.05 gr. An Adult has about 7000-ml blood. If the entire quantity of CHG contained in GEL-X is absorbed in the blood, it would reach 7.1 P.P.M, which is less than $10^{-2}$ of the safety levels as expressed in the above two researches.

Bentwich and Insler (68) tested blood residues, as described in chapter 21 "Clinical research on Gel-X™" in this article. The test was performed to find residues in levels of P.P.B. Although the research showed penetration of negligible amounts in 40% of the volunteers, after 24 hours - no detectable levels could be found in the blood, and it was concluded that the negligible residues were taken out in the Urine.

### 13. Effects on Vaginal Flora

Researches have confirmed that vaginal flora returns to itself after Chlorhexidine Gluconate cream has been inserted into the vagina.

Easmon et al (36) show in their research that 4 hours after the insertion of Chlorhexidine cream into the vagina, the maximum reduction of flora was reached. Later, the flora starts to return, and after 6 hours it starts to grow logarithmically.

The same results are shown by Dykes et al (35) with the use of Chlorhexidine solution. Passloer (54) in his research on disinfectants in the vagina, verifies that when the use of chlorhexidine in the vagina is stopped, the flora returns to itself.

Jackson et al (55) proved in his research that after 18 hours, the flora was identical to the flora found prior to the use of Chlorhexidine disinfectant. Women who had been treated with a placebo, and had received the same disinfectant treatment, showed the same results.

Shubair et al (50) conducted their research on the effect of Chlorhexidine Gluconate douche on normal vaginal flora. 20 Volunteers were treated daily with a solution containing 0.5% Chlorhexidine Gluconate (the same concentration of CHG as in GEL-X) for 7 consecutive days 180 ml. was used for this purpose, (whereas the maximum amount in GEL-X is 10 ml).

No side effects were observed, even after 30 days. The natural flora at the end of the research was nearly the same as at the beginning. Fungi which were found, were not different, and prevalence was not significantly increased at the end of the study. No cases of vaginitis, either yeast vaginitis or bacterial infection, were definitely diagnosed as arising as a consequence of the use of Chlorhexidine Gluconate. No untoward effects on the participants were observed.
For additional information, refer to Chapter 20 “Gel-X replaces the absence of the vaginal natural flora during action.”

14. Adverse effects of Nonoxynol-9 and Benzalkonium Chloride.

Nonoxynol-9 (NX-9) and Benzalkonium Chloride (BZC) are commonly used ingredients in spermicidal products. Well conducted clinical trials, show a protective effect of these spermicides against STD’s bacteria, parasites and viruses. Nevertheless, these spermicides may cause a potential risk to users due to the chemical irritation of the vaginal epithelium (80).

Kreiss et al (81), while assessing the efficacy of NX-9 preventing HIV transmission on Nairobi prostitutes, failed to demonstrate the efficacy of NX-9, as it was associated with an increased frequency of higher incidence of genital ulcers and of genital ulcer-associated seroconversion, and in another research (82), to determine the efficacy of the NX-9 contraceptive sponge in preventing sexual acquisition of the HIV in Nairobi prostitutes. The author concluded that genital ulcers and vulvitis occurred with increased frequency. (This, in our opinion, was due to the chemical irritation of the vaginal epithelium, or increased fungal growth in the absence of the natural flora). Therefore, they were unable to demonstrate that the NX-9 sponge was effective in reducing the risk of HIV infection amongst highly exposed women.

Martindale (79) page 786 defines that “BZC inactivates HIV in vitro. This property may contribute to the prevention of sexually transmitted disease when BZC is used as a spermicide. NX-9 has similar activity and in discussing that spermicide the view has been expressed that undetected low grade epithelial or mucosal damage arising from spermicide use may render the tissues more permeable to HIV”.

15. Side Effects of Chlorhexidine

During the 30 years of use of chlorhexidine in various medical fields, some cases of side effects have been published in the literature. These include ototoxicity and deafness on contact of chlorhexidine with the middle ear, conjunctivitis associated with the use of soft contact lens solution containing chlorhexidine, one case of colitis after a cleanser enema and one case of gastritis induced by repeated self ingestion of a 4% chlorhexidine gluconate solution (40).

As was discussed earlier, chlorhexidine is used extensively in dental practice as a mouth rinse for inhibiting plaque and as a treatment for gingivitis. The common side effects reported are disturbance of taste sensation which can last from few minutes to several hours, gingival bleeding and epithelial cell desquamation. A case of swelling of the lips after rinsing with 0.2% chlorhexidine solution was also reported. However, histological examinations of human mucosal biopsies taken after 18 months of daily exposure to chlorhexidine did not reveal any adverse effects in the oral mucous (25).

A more severe side effect associated with the use of chlorhexidine is anaphylactic shock. Moghadam et. Al (39) found in the English language literature eight such cases: six in Japan, one in Sweden and one in Australia, although Ohtosi et. al (41) state that 30 cases of anaphylactic shock cases have been reported in Japan in the period between 1974 and 1984.
These authors examined the sera of eight of the anaphylactic shock patients and found increased levels of IgE antibody as detected by radioallergosorbent technique (RAST). They suggest that IgE antibody specific to chlorhexidine is the mediator of the allergic reaction.

Some few cases of contact hypersensitivity to chlorhexidine have been reported in the literature (21,39,42), but the sensitizing potential of chlorhexidine has been disputed, probably due to some uncertainty in the interpretation of patch tests, positive reactions being considered irritant and not allergic (24).

In order to assess the skin sensitivity to chlorhexidine, a study (24) was conducted in Denmark on 551 patients suffering from skin infections between 1978 and 1981. Positive reactions were obtained in 14 patients, 10 of which suffered from venous or traumatic ulcers of the leg.

However, these results should be treated with some reservation as a later joint study (43) performed by 14 Danish dermatologists on 2061 patients with eczematous lesions and using patch tests showed that 48 patients gave positive reactions.

Out of the 48 patients, 14 were retested six months later and only one patient showed positive reaction. The authors suggested that the apparent loss of sensitivity may be due to irritable skin at the initial testing, the so-called "excited skin syndrome", and concluded that these findings indicate that the sensitizing potential of chlorhexidine is very low.

On March 1998, FDA has issued a public notice (84) regarding Hypersensitivity reaction to CHG. “Although the antimicrobial properties of chlorhexidine are well known, it is not as well known that chlorhexidine has been associated with hypersensitivity reactions. Anaphylactoid and other types of reactions have been reported with chlorhexidine used topically, intrarethraly, as a lubricant on urinary catheters, and with chlorhexidine-impregnated catheters. These incidents have occurred in Japan, Switzerland, the United Kingdom, Australia, Malaysia, and the United States.

Immediate systemic hypersensitivity reactions to chlorhexidine gels/lubricants used during urological procedures: Hypersensitivity reactions associated with chlorhexidine gels/lubricants used during urological procedures have been reported in several countries. (None have been reported thus far in the U.S.) In one case, a 61 year-old man in the Netherlands exhibited a severe allergic reaction associated with a chlorhexidine gel used for an intra-urethral preparation. In another incident in Netherlands, Australia, a 52 year-old man had an anaphylactic reaction to a chlorhexidine lubricant on a urinary catheter while undergoing a temporal lobectomy. Six cases of severe allergic reactions to chlorhexidine gel used with urinary catheters have also been reported in Melbourne, Australia.”

In view of the very extensive use of chlorhexidine as a hand wash, preoperative disinfectant, wounds and ulcers cleanser, oral rinse against plaque and gingivitis, vaginal wash for treating vaginitis, and in numerous preparations as lozenges, lotions, gargles, disinfectors, agents for purulent infections, topical astringents, topical antiphlogistics, ectoparasiticides, emollients and tooth paste (39), for over 40 years, the low incidence of side effects indicates that chlorhexidine is quite a safe agent.
16. Virucidal Activity of Chlorhexidine

Chlorhexidine, beside its microbicidal properties, is also an effective antiviral agent on enveloped viruses, rotaviruses and hepatitis B.

A study (9), designed to assess the in vitro effectiveness of 0.12% chlorhexidine gluconate solution, used as a mouthrinse, against Herpes Simplex virus (HSV), Cytomegalovirus (CMV), Influenza A virus, Parainfluenza, Polio and Hepatitis B virus (HBV), showed that chlorhexidine gluconate, at a concentration of 0.12%, is highly effective against enveloped viruses. **After 30 seconds**, it reduced HSV titer by 97%, CMV titer by more than 99.7%, Influenza A by 93%, Parainfluenza by 59% and HBV by 85%. Only the Poliovirus, a non-enveloped virus was not affected.

**After 5 minutes** exposure, HSV, CMV and Influenza A were completely inactivated while Parainfluenza and HBV showed reductions of 91% and 94% respectively in virus titers.

This study (9) suggests that chlorhexidine gluconate is also effective against rotavirus which is a non-enveloped virus containing surface glycoproteins that are essential for infectivity. This is probably due to chlorhexidine gluconate interaction with the surface glycoproteins.

Modak S, et al (69), concludes that CHG gloves rapidly inactivated, in vitro and in vivo, retrovirus and hepatitis B virus.

Pauwels and Clercq (76), conducted research regarding various compounds, including CHG, which could be considered to be Microbicides, preventing heterosexual transmission of HIV. It was concluded, that in particular, combination of two compounds seems to be an attractive approach.

Harrison and Chantler (77) examined, in vitro, the effect of CHG and NX-9, either singly or in combination, on the replication and infectivity of HIV and the survival of both lymphocytes (MT2 cells) and human spermatozoa. 0.1% wt/vol. of CHG for 1 minute destroyed the MT2 viability. 1 mg/ml (0.1% wt/vol.) CHG was 80%-100% effective in inactivating HIV which was estimated by the output of p24 (the HIV core protein) and the concentration of virus was determined by titration with MT2 cells. Contrary or additionally to Pauwels (76), certain combinations of NX-9 and CHG were antagonistic in their inactivation of HIV.

Chlorhexidine virucidal activity is manifested by inhibition of virus replication and cytolitic activity, as was indicated by Park and Park (13) who evaluated the in vitro inhibiting activity of chlorhexidine gluconate in concentrations ranging from 0.0005% to 0.2% on Herpes Simplex virus (HSV-1). After 10 minutes exposure of the HSV-1 to 0.1% solution of chlorhexidine gluconate, the virus titer was reduced by 50% while a 0.2% solution reduced the virus titer by 70%. The cytolitic activity of the HSV-1 was also markedly inhibited after 10 minutes treatment with Chlorhexidine.

These authors, in an in vivo research (13), also found that 0.2% solution of chlorhexidine gluconate applied topically to the HSV-1 infected foreskin of mice 2 hours after infection, moderately but significantly inhibited the development of viral lesions and reduced virus titer in the skin and trigeminal ganglia of the mice.
Park et. al. (14) found that, beside its direct virucidal activity, chlorhexidine also inhibits the synthesis of viral DNA at concentrations low enough not to interfere with the cellular DNA synthesis of the host cell.

Other in vitro antiviral activity of chlorhexidine gluconate against the Human Immunodeficiency virus (HIV) was demonstrated by the following two studies. In the first one (15), the HIV was completely inactivated after exposure for 15 seconds to a 1:100 diluted solution of 4% chlorhexidine gluconate and 4% Isopropyl alcohol (concentrations of 0.04% and up). Chlorhexidine gluconate, acting alone, completely inactivated the HIV at concentrations of 0.05% and above. The second study (16) showed that replication of HIV was inhibited by chlorhexidine gluconate at concentrations of 0.2% or greater.

In an in vitro and in vivo infectivity test of gloves containing chlorhexidine gluconate (69). The CHG gloves were tested to determine their ability to rapidly inactivate infectious pathogens, (e.g., bacteria, fungi, parasites and viruses, as well as lymphocytes and macrophages that are known to be the primary carries of H.I.V), that may permeate or leak through latex surface, (same material as condoms). CHG gloves rapidly inactivated all the pathogens tested including retrovirus and hepatitis B virus (90% to 100%). Live viruses were not detected in any of the CHG group compared to 26% detected in the control group. It was concluded that the use of CHG may reduce the risk of exposure to infectious fluid-borne pathogens. Tests have also shown that CHG coating does not alter physical properties of the glove (latex, same as condoms), furthermore, CHG gloves do not show potential for dermal irritation or sensitization.

Wood A, Payne D (79) assessed the antiviral action of chlorhexidine against a range of enveloped human viruses. The results indicated that CHG as well as other antiseptics were effective in inactivating the enveloped viruses HSV-1 and HIV-1. CHG also proved to be effective as antiviral, within 1 min on HIV in the presence of whole human blood.

Kawana R, et. al. (71) tested in vitro the inactivation of a range of viruses. CHG inactivated rubella, measles, mumps and HIV viruses within a short period of time. Rotavirus were inactivated by povidone-iodine, Benzalkonium and benzethonium chlorides. Unfortunately, the two mentioned chlorides are not permissible for use as vaginal Microbicides (67).

Biggar RJ, et. al. (72), of the Viral Epidemiology Branch, National Cancer Institute, Bethesda, Maryland, U.S.A, examined the efficacy of birth canal washing against perinatal transmission of 3637 women. The intervention consisted of washing the birth canal with 0.25% Chlorhexidine,. Intervention was performed on admission in labour and thereafter, every 4 hours until delivery. No adverse reactions to the intervention procedure were seen. As 2094 (30%) of the enrolled women (3327 of the control women + 3637) were HIV infected, 59% of their infants were seen in follow-up. 25% of the intervention group vs 39% in the control group, of the infants born to membrane ruptured women of more than 4 h before delivery, were infected. A difference of 56% compared to the overall comparison between the two groups who did not show a significant impact. (These results were opposed when the intervention was for three months (73)). The authors interpret that if birth canal exposure is an important risk factor, different or additional methods should be tested or that perhaps birth canal exposure is not a major contributtor to prenatal infection risk.
Taha TE, et. al. (73) of John Hopkins University, Baltimore MD, USA, performed a clinical in vivo trial on 6965 women giving birth over a six month period, where 3635 women received intervention after two months of no intervention. This was followed by three months of intervention and a final month of no intervention. The intervention was a manual wipe of the maternal birth canal with 0.25% chlorhexidine solution. Hitherto, there were no adverse reactions related to the intervention among the mothers or their children. In this research, as opposed to Biggar RJ, et. al. (72), the conclusions were: Cleansing the birth canal with chlorhexidine reduced early neonatal and maternal postpartum infection problems. The safety, simplicity and low cost of the procedure suggest that it should be considered as standard care to lower infant and maternal morbidity and mortality.

17. Chlorhexidine as a Spermicide

During coitus, ejaculation deposits a pool of semen in the posterior vaginal formix in close contact with the cervical mucus. As a result, the sperm, upon liquefaction of the seminal coagulum, may penetrate rapidly into the mucus with little direct contact with the vaginal fluids (17).

Thus, a Spermicide must have a dual action in order to be an effective one. First it must have a direct spermicidal effect on the spermatozoa, inhibiting their motility, or movement toward the mucus. Secondly, it must affect the cervical mucus in such a way as to prevent the penetration of motile sperm into the mucus.

In a study (17) aimed at evaluating the in vitro spermicidal effectiveness of chlorhexidine, it was found that exposure of sperm for 3 minutes to 0.5 mg/ml of chlorhexidine resulted in total loss of sperm motility.

Louis and Pearson (10) investigated the in vitro effect of chlorhexidine on sperm motility using the Trans-membrane Ratio technique. In this technique the number of chlorhexidine treated sperm crossing a membrane during 2 hours incubation period is compared to the number of untreated sperm that crossed the membrane. The test was conducted at various chlorhexidine concentrations and showed that 0.1 mg/ml of chlorhexidine reduced sperm motility by 40% and 0.5 mg/ml reduced motility by 60%. At 0.215 mg/ml, motility was reduced by 50% (EC50). For comparison, the EC50 for nonoxynol-9, a widely used Spermicide, was 0.215 mg/ml.

Chijioke et. al. (18) tested the in vitro reduction of sperm motility by chlorhexidine diacetate using the Sander-Cramer test. This test involves finding by visual observation the lowest concentration of a Spermicide that will abolish sperm motility within 20 seconds. The minimum concentration of chlorhexidine diacetate that was required for complete inhibition of sperm motility was found in this study to be 4.81 mg/ml.

Another approach for evaluating the effect of chlorhexidine on sperm was taken by Sharman et. al. (17). These authors compared qualitatively the propagation rate of sperm in a column filled with chlorhexidine treated cervical mucus against an untreated control. The concentrations used were between 0.1 mg/ml and 10 mg/ml. It was found that there was a highly significant inhibition of sperm entry into the mucus at all concentrations and durations of the test and all the sperm was immotile at the time of observation.
The authors conclude that chlorhexidine can immobilize sperm in mucus at concentrations which are lower than those known to be spermicidal when applied directly to semen. They suggest that chlorhexidine prevent migration of sperm into the cervical mucus by altering the mucosal structure. This hypothesis was corroborated by a later study (18) which indicated structural changes in chlorhexidine treated mucus as evidenced by numerous tests.

Harrison and Chantler (77) who examined, in vitro, the effect of CHG and NX-9, either singly or in combination, concluded, among other conclusions, that CHG in concentration of 2.063 mg/ml, (0.206% wt/vol.) completely immobilized the sperm after one minute.

An in vivo study (19) conducted on primates (the stump tail macaque) showed that polyvinylethanol films containing 62.5-75 mg of chlorhexidine diacetate, which were inserted into the vagina next to the cervix prior to coitus completely inhibited sperm motility. At lower doses (12.5-15 mg and 25-30 mg) inhibition of motility was partial.

Thus, chlorhexidine is not only a safe and effective antibacterial agent, it is also an effective virucide and spermicide. According to the criteria set forth in the preceding discussion, chlorhexidine can be considered as a suitable added chemical barrier for prevention of infection by sexually transmitted diseases including infection by HIV.

18. Gel-X™

GEL-X has the following properties:-

- Provides proper lubrication, thus avoiding torn blood vessels.
- Destroys bacteria and viruses such as HSV, CMV, Influenza A, Parainfluenza, Hepatitis B and HIV. (9,10,13,14,15,16,31,32,35,56,57,58,67,69,71,72,73,74,76,78)
- Inhibits the mobility of sperm cells. (10,17,18,19,77)
- Prevents the penetration of sperm cells into the uterus by changing the cervical mucous. (17,19)
- Prevents fungi from developing in the vagina in the absence of natural flora. (31,35,36,37)
- Facilitate artificial lubrication in case of vaginal dryness, atrophy and sensitivity.
- Safe for use, with no known side effects. (21,24,39,40,42,43,67,68,72,73)
- Can be easily washed away by water after use.

Any vehicle designed to spread chlorhexidine onto the genital organs should be able to adhere to them and not be rubbed off easily.

Pauwels and Clercq (76), conducted research regarding various compounds, including CHG, which could be considered to be Microbicides, preventing heterosexual transmission of HIV. It was concluded, that in particular, combination of two compounds seems to be an attractive approach. Gel-X™ is also a mixture of two antiseptic compounds, CHG to destroy the STD’s and Paraben as a fungicide.
In a study, Kollee et. al. (44) applied an aqueous gel based on hydroxy propyl methyl cellulose and glycerol to the vagina of 17 women and found that the viscous gel adhered to the vaginal wall. Gel-x™ is based on an aqueous gel containing hydroxyethylcellulose (Natrosol™) a similar gelling material.

Runganga AO, Kasule J (75) concludes that from the point of view of AIDS prevention, expectations of a dry and contracted vagina in sexual intercourse may reduce acceptability + use of condoms. Gel-X™, which serves as an antiseptic lubricant can lubricate and prevent transmission as well.

The composition of Gel-x™ is very similar to K-Y® Lubricating Jelly which is manufactured by Johnson & Johnson and listed in Martindale (45). Gel-x™ contains, beside Natrosol™, Glycerin and Propylene Glycol, which act as lubricants, Chlorhexidine as a disinfectant and Methylparaben as a fungicide.

An informal survey conducted among call girls in Israel indicated that most of them are using the K-Y jelly as a lubricant prior to sexual relations.

Landesman et al (78) evaluated the relation of obstetrical factors and other variables to the prenatal transmission of HIV-1 on 525 infected women. It was concluded that ruptured membranes increased the transmission of HIV whether the rupture occurred more than 4 hours or less than 4 hours before delivery. It can be concluded, that lubricated vagina prior to intercourse, will avoid ruptured vaginal membranes, thus avoiding the transmission of HIV to the partner.

Pollak Ltd. has been manufacturing for over 20 years similar products with the same components for medical lubrication of devices inserted into the urethra, vagina and vulva. The product, called "Pedicat", is used and distributed world wide, and has F.D.A approval since 1981.

The viscous nature of Gel-x™, as well as its lubricating properties, will reduce over friction of the genital organs during coitus, thus reducing rupture of small blood vessels and the chances of transmission of sexually transmitted diseases (STD’s), including HIV.

A study (7), cited previously, found that while unruptured condoms, tested in vitro, inhibit transmission of viruses completely (in 10 out of 10 condoms) when condoms were artificially ruptured, transmission was evident in seven out of ten.

When applied on condoms, the above mentioned properties of Gel-x™ will reduce markedly the incidence of condom rupture and increase their effectiveness as physical barriers against infection by STD. Moreover, as Gel-x™ is an aqueous gel, it will not decrease the physical properties of latex condoms, as opposed to petrolatum based gels which have a detrimental effect on the strength and other properties of latex.

When Gel-x™ is being applied on the penis or the vaginal wall, some 5 ml are being used. As the typical fluid volume in the vagina after ejaculation might total 4-10 ml, the expected dilution will be 2 to 3 fold.

Studies mentioned earlier showed that chlorhexidine is virucidal at concentrations ranging from 0.04% to 0.2%. The chosen concentration of 0.5% chlorhexidine in Gel-x™ will give, after dilution with the fluids in the vagina, a concentration of 0.16% to 0.25%, well within the range of its virucidal concentration.
19. Anti-HIV activity of Gel-X

In-Vivo Test to determine the anti-HIV activity of Gel-X was conducted on behalf of the Medical Research Council, (MRC) UK, by Dr. Robin J Shattock, Ph.D. Senior Lecturer in Cell Biology, of the Department of Infectious Diseases, St George’s Hospital Medical School, London U.K.

Materials used were:
Gel-X formulation.
The cell line C8166 was supplied by MRC Aids Reagents Programme.

Methods

Culture of Cell line C8166 and virus HIV-1 RF:
Cells were cultured in RPMI containing 10% foetal calf serum (FCS), L-Glutamine and Penicillin and Streptomycin. Cells were passaged every 3-4 days. Prior to use, cells were centrifuged and re-suspended in fresh media at 4x10^5 cells/ml. Virus was grown in the cell line CEM, harvested after 7-10 days, filtered and stored at 70°C. Virus was determined to have a TCID_50 of 3x10^10/ml on C8166 cells.

Poly-L-Lysine capture assay:
Wells of 96-well tissue culture plate were coated with 100 µl of poly L-lysine (PLL) at 50 µg/ml for 1 hour at room temperature. After 2 washes with phosphate buffered saline (PBS), 50 µl virus (HIV-1RF approx. 1x10^9 virus particles/ml) was added for a further hour. Wells were washed twice again with PBS followed by the addition of a 10-fold serial dilution of Gel-X. Where indicated Gel-X was mixed with either 20% seminal plasma or 10% blood. After 1 hour at room temperature, wells were washed 4 times with PBS to ensure removal of all the Gel-X and 200 µl C8166 cells were then added to each well. Plates were cultured for 7 days in a humidified atmosphere at 37°C with 5% CO_2.

Viral infection was determined by measurement of reverse transcriptase activity in the supernatants.

Results

Gel-X was virucidal (i.e. inactivated the virus) when used neat or 1/10. However, the virucidal activity of Gel-X was reduced in the presence of seminal plasma or blood, only providing 100% protection when used neat (i.e. 0.5% chlorhexidine) (Table II). Despite repeat washing, when used neat, sufficient Gel-X remained bound to the culture plates to be toxic to the indicator cells, demonstrating a lack of selectivity of the agent between viral and cellular membranes. No toxicity was observed at all when Gel-X was diluted above 1/10.
Table II: Virucidal activity of Gel-X in presence of seminal plasma or blood.

<table>
<thead>
<tr>
<th>Gel-X</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>neat</td>
<td>34</td>
<td>50</td>
<td>865</td>
<td>702</td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>1:10</td>
<td>46</td>
<td>43</td>
<td>5260</td>
<td>1872</td>
<td>14436</td>
<td>4332</td>
</tr>
<tr>
<td>1:100</td>
<td>26379</td>
<td>2746</td>
<td>7326</td>
<td>1966</td>
<td>13397</td>
<td>2305</td>
</tr>
<tr>
<td>1:1000</td>
<td>23972</td>
<td>2285</td>
<td>11371</td>
<td>2727</td>
<td>15156</td>
<td>1057</td>
</tr>
<tr>
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<td>25425</td>
<td>2794</td>
<td>27254</td>
<td>5414</td>
<td>25049</td>
<td>1125</td>
</tr>
<tr>
<td>1:100000</td>
<td>12855</td>
<td>2293</td>
<td>34285</td>
<td>7484</td>
<td>20038</td>
<td>1255</td>
</tr>
<tr>
<td>Virus control</td>
<td>0</td>
<td>21138</td>
<td>2685</td>
<td>16859</td>
<td>4513</td>
<td>18541</td>
</tr>
</tbody>
</table>

Conclusion:

These results demonstrate that Gel-X is active against HIV when used neat or 1/10. These findings in agreement with previous studies demonstrating that chlorhexidine has an IC\textsubscript{50} value (50% inhibitory dose) of 0.25 µM, a CC\textsubscript{50} value (50% toxic dose) of 1.3µM and a selectivity value of 5.2 (Int J STD & AIDS). The effects of semen and blood on the anti-HIV activity of Gel-X are similar to those reported for the anti-bacterial effects of chlorhexidine (Sex Trans Diseases 2000; 27:74-78).

In summary, Gel-X is likely to inactivate cell free and cell associated HIV in vivo.

20. Compatibility of Gel-X™/CHG with latex condoms

In order to test the reaction and compatibility of Latex condoms with Gel-X™ an assortment of condoms were chosen.

Test results demonstrated that Gel-X™ had no adverse effects on any type of the condoms. Latex is the ingredient gloves and condoms are made of. Modak et al (69), tested CHG coated latex gloves, and concluded in their research that CHG coating does not alter physical properties of the glove. Furthermore, CHG gloves do not show potential for dermal irritation or sensitization.

21. Gel-X™ replaces the absence of the vaginal natural flora during action.

Many OTC vaginal spermicides marketed today do not specify time limitation for their use. In these cases, vaginal flora will consequently be absent for extended periods of time. The fact that there are no limitations for use of these products, vaginal spermicides can be and in many cases are used frequently, - even daily. To the best of our knowledge, and due to absence of this subject from the published literature, it can be concluded therefore, that the absence of natural vaginal flora is not a cause for concern when spermicides are used.

However, when a product is intended for use as a microbicide, a regular spermicide is not sufficient protection. In the absence of natural flora, fungal growth can occur,
creating ulceration, which allows for the penetration of STD’s. Similarly, if the spermicide chemically destroys vaginal tissue, the same phenomenon can occur.

The fact is appreciated that CHG is a surfactant and does not chemically affect the vaginal tissue (49, 67). It has both advantageous antiseptic properties and spermicidal properties. It also destroys the natural bacterial flora (mainly the Lactobacillus) in the vagina which regulate and prevent the growth of fungi (Candida). By destroying the natural flora, fungal growth results and this causes ulcers, which expose the blood vessels to infection. Such ulcers in the vagina enable STD’s to penetrate and enter the bloodstream, so it is vital that the formation of these ulcers be prevented.

Two consecutive researches were conducted, to determine the influence of Microbicides (CHG, NX-9 and BZC) and fungicides (Methylparaben, Miconazole Nitrate) and mixtures of the abovementioned Microbicides and fungicides, on natural vaginal flora – in particular; Lactobacillus, Staphylococcus Aureous as bacteria and Candida Albicans as a fungus.

The researches were to prove that when commonly used Microbicides are introduced to the vagina without a fungicide, fungi will grow, but when they are introduced together with a fungicide, the growth is prevented. The methodology was to inoculate 1ml each of a solution containing 100,000 microorganism (in the second - for each one, 63 plates + 1 control) in a hollow depression of 8 mm, and to add to each hollow 0.05ml of each solution, and to incubate for 48 hours. Later, measurements were taken from the lip of the hollow, up till the start of microbial growth.

The research was conducted in our laboratories in conjunction with Migal Galilee Technological Center, Kiriat-Shmoneh, with the assistance of our Pharmacist, Abraham Shimberg M. Sc. And Dr. Evelyn Hammerschlag, Microbiologist, of Migal.

Table III: Measurements of empty zone readings following microbiological growth after 48 hours.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Mixture *</th>
<th>Staphy. Aureaus</th>
<th>Candida Albicans</th>
<th>Lactobacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 mm</td>
<td>0 mm</td>
<td>0 mm</td>
<td>0 mm</td>
</tr>
<tr>
<td>CHG 0.5% + Methylparaben 1%</td>
<td>13 mm</td>
<td>17 mm</td>
<td>15 mm</td>
<td>13 mm</td>
</tr>
<tr>
<td>CHG 0.5%</td>
<td>10 mm</td>
<td>6 mm</td>
<td>5 mm</td>
<td>11 mm</td>
</tr>
<tr>
<td>BZC 1%</td>
<td>11 mm</td>
<td>5 mm</td>
<td>4 mm</td>
<td>16 mm</td>
</tr>
<tr>
<td>CHG 0.5% + Miconazole Nitrate 1%</td>
<td>3 mm</td>
<td>1 mm</td>
<td>15 mm</td>
<td>8 mm</td>
</tr>
<tr>
<td>NX-9 12.5%</td>
<td>5 mm</td>
<td>5 mm</td>
<td>0 mm</td>
<td>11 mm</td>
</tr>
<tr>
<td>BZ 1% + Methylparaben 1%</td>
<td>11 mm</td>
<td>18mm</td>
<td>16 mm</td>
<td>14 mm</td>
</tr>
<tr>
<td>NX-9 12.5% + Propylbenzoate 1%</td>
<td>1 mm</td>
<td>7 mm</td>
<td>10 mm</td>
<td>8 mm</td>
</tr>
</tbody>
</table>

* Mixture = Natural flora microorganisms.

The tables clearly demonstrate that when using mixtures of vaginal Microbicides and Fungicides, or fungicides by themselves, the Candida Albicans (most common vaginal fungus) is destroyed, whilst on vaginal Microbicides which did not contain fungicides, the Candida
Albicans was not destroyed. On the other hand, fungicides by themselves did not destroy the Lactobacillus and Staphylococcus Aureaus.

Table IV: Average measurements (of 7 each) of the empty zones reaching following growth in mm. after 48 hours.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Staph. Aureus</th>
<th>Candida Albicans</th>
<th>Lactobacillus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 mm</td>
<td>0 mm</td>
<td>0 mm</td>
</tr>
<tr>
<td>CHG 0.5% + Methylparaben 1%</td>
<td>17.0 mm</td>
<td>14.0 mm</td>
<td>14.4 mm</td>
</tr>
<tr>
<td>CHG 0.5%</td>
<td>9.9 mm</td>
<td>3.1 mm</td>
<td>14.3 mm</td>
</tr>
<tr>
<td>BZC 1%</td>
<td>6.1 mm</td>
<td>2.1 mm</td>
<td>17.3 mm</td>
</tr>
<tr>
<td>CHG 0.5% + Miconazole Nitrate 1%</td>
<td>7.3 mm</td>
<td>15.6 mm</td>
<td>14.4 mm</td>
</tr>
<tr>
<td>NX-9 12.5%</td>
<td>4.9 mm</td>
<td>0.6 mm</td>
<td>10.7 mm</td>
</tr>
<tr>
<td>BZ 1% + Methylparaben 1%</td>
<td>8 mm</td>
<td>16.4 mm</td>
<td>17.1 mm</td>
</tr>
<tr>
<td>NX-9 12.5% + Propylbenzoate 1%</td>
<td>2.1 mm</td>
<td>12.7 mm</td>
<td>10.1 mm</td>
</tr>
<tr>
<td>Methylparaben 1%</td>
<td>1.1 mm</td>
<td>18.6 mm</td>
<td>2.1 mm</td>
</tr>
<tr>
<td>Miconazole Nitrate 1%</td>
<td>0.3 mm</td>
<td>16.4 mm</td>
<td>2.3 mm</td>
</tr>
</tbody>
</table>

Therefore, Gel-X™ comprises the combination of:
- Lubricant to reduce friction, and thereby tissue irritation.
- Specific antiseptic (CHG), which destroys bacteria and viruses and immobilizes sperm cells and reacts with the cervical mucosa to prevent the penetration of sperm cells.
- A fungicide (Methyl-Paraben), to prevent the development of fungi in the absence of the natural vaginal flora.

Clinical tests (68) demonstrated the effectiveness of the composition, and have verified that no ulceration occurs, even though the natural flora is destroyed.

22. Clinical research on Gel-X™

A clinical research (68) (Phase III) using GEL-X, conducted in the Kaplan Hospital, Rehovot (near the Weizman Institute) Israel, on 20 healthy, non pregnant women volunteers between the ages of 18 and 55 followed by colposcopy inspection, showed the undermentioned results:-

Examination of the vagina at all stages, resulted in No Pathological Findings in any of the women. No changes in vaginal tissues were found, and there was no evidence of irregularities pertaining to sexual organs after use of GEL-X. All volunteers felt well, and none complained of any ill effects. Only one volunteer reported a slight burning sensation for the first 10 minutes after which this feeling subsided. This volunteer had disclosed to the gynecologist, at the outset, that she was sensitive to some ointments. This was the only case of a volunteer expressing any discomfort.

In order to ascertain residues in the blood, blood was tested in HPLC to determine levels in P.P.B.(ng/ml)
a) All volunteers showed no detectable levels (>20 P.P.B.) at Time 0.

b) At Time + 1 Hour, (after a minimum of 1 hour and not more than 2 hours after the injection of GEL-X into the vagina), 12 volunteers showed no detectable levels (>20 P.P.B.), whereas the levels detected in the blood of the remaining 8 volunteers ranged from 25 to 94 P.P.B.

c) At Time + 24 Hours, (24 hours after the insertion of GEL-X into the vagina, and 22 hours after the dilution of GEL-X with 10 cc physiological saline from the vagina), no detectable levels (>20 P.P.B.) were found. All P.P.B's negligible residues were taken out in the Urine, see also Brougham et. al. (51)

The Microorganisms that were found at Times 0, were destroyed after the insertion of Gel-x™ that demonstrates the efficacy in the vagina. 24 hours later, the normal vaginal flora was observed and specially the Lactobacillus.

The foregoing indicates that Gel-x™, when present in the vagina or spread onto the erect penis (with or without a condom) prior to intercourse, will reduce infection by STD's, due to its lubricating, antimicrobial, virucidal and spermicidal properties, and no other side effects were observed.

It can also be concluded that the effectiveness of condoms as physical barriers against transmission of sexually transmitted diseases will be enhanced by applying Gel-x™ onto the outer skin of condoms prior to sexual relations.
Table V:
Clinical researches (in vivo) regarding safety of Chlorhexidine and Gel-X™

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Con. %</th>
<th>Research</th>
<th># in Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral use</td>
<td>2.0</td>
<td>Park JB. et al</td>
<td>13</td>
</tr>
<tr>
<td>Oral, slow release for 7 days</td>
<td>3.0</td>
<td>Hildebrandt et. al</td>
<td>61</td>
</tr>
<tr>
<td>Oral use, 2 decades of OTC use.</td>
<td>0.2-2.0</td>
<td>Albandar et. al</td>
<td>60</td>
</tr>
<tr>
<td>Applied to the Penis</td>
<td>4.0</td>
<td>Sanderson PJ et al</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gilmore DS et al</td>
<td>30</td>
</tr>
<tr>
<td>Microflora in the vagina</td>
<td>4.0</td>
<td>Vorherr et. Al</td>
<td>31</td>
</tr>
<tr>
<td>Whole body</td>
<td>4.0</td>
<td>Sanderson and Haji</td>
<td>32</td>
</tr>
<tr>
<td>Vagina</td>
<td>0.2-4.0</td>
<td>Miscellaneous</td>
<td>34,35,36,37,38</td>
</tr>
<tr>
<td>Vagina, 2238 women, 60ml CH 2g/l . 2 to 6 consecutive times</td>
<td>0.2</td>
<td>Burman et al</td>
<td>47</td>
</tr>
<tr>
<td>Vagina, 2853 women.</td>
<td>0.2-1.0</td>
<td>Henrichsen et. al</td>
<td>64</td>
</tr>
<tr>
<td>Birth canal for 3 consecutive months on 3635 women giving birth</td>
<td>0.25</td>
<td>Taha TE, et. al.,</td>
<td>73</td>
</tr>
<tr>
<td>Birth canal of 3637 women giving birth</td>
<td>0.25</td>
<td>Biggar RJ, et. al.,</td>
<td>72</td>
</tr>
<tr>
<td>Vagina, Vaginal flora &amp; residues in P.P.B of 180 ml daily. 7 consecutive days</td>
<td>0.5</td>
<td>Shubair et al.</td>
<td>50</td>
</tr>
<tr>
<td>Safe with latex condoms</td>
<td></td>
<td>Modak S, et. al.</td>
<td>69</td>
</tr>
<tr>
<td>Human vaginal membrane</td>
<td>1.0</td>
<td>Spitzbart - H</td>
<td>63</td>
</tr>
<tr>
<td>Burns treated topically</td>
<td>2.0</td>
<td>Brougham et al</td>
<td>51</td>
</tr>
<tr>
<td>Vaginal residues</td>
<td>0.2</td>
<td>Nilsson et al</td>
<td>25</td>
</tr>
<tr>
<td>Vaginal flora</td>
<td>0.2-4.0</td>
<td>Miscellaneous</td>
<td>36,35,54,55</td>
</tr>
<tr>
<td>Skin sensitivity on 551 patients</td>
<td>1.0</td>
<td>Sanderson PJ et al</td>
<td>28</td>
</tr>
<tr>
<td>Skin sensitivity on 2061 patients</td>
<td>1.0</td>
<td>Bechgaard E et al</td>
<td>43</td>
</tr>
<tr>
<td>Vagina</td>
<td></td>
<td>Martindale 30 ED.</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Page 789/3</td>
<td></td>
</tr>
<tr>
<td>Does not disrupt the vaginal epithelium, not a surfactant</td>
<td>1.0</td>
<td>Editorials, The Lancet Chantler E</td>
<td>49 48</td>
</tr>
<tr>
<td>Vagina, Clinical research on Gel-x™</td>
<td>0.5</td>
<td>Bentwitz et al</td>
<td>68</td>
</tr>
<tr>
<td>Veins, 96 hours CH catheter in vein</td>
<td></td>
<td>Carruth W A et. al.</td>
<td>59</td>
</tr>
</tbody>
</table>
Table VI:
Clinical researches regarding efficacy of Chlorhexidine and Gel-X™

<table>
<thead>
<tr>
<th>Microorganism and/or tissue</th>
<th>Research</th>
<th># in Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial agent with a broad spectrum activity</td>
<td>British Pharmacopoeia 1980(I)</td>
<td>8</td>
</tr>
<tr>
<td>Variety of bacteria: Gram positive Gram negative, aerobes &amp; anaerobes</td>
<td>Bernstein et. al.</td>
<td>9</td>
</tr>
<tr>
<td>Antibacterial effect causing an alternation or breakdown of bacteria structure</td>
<td>Louis S M et. al.</td>
<td>10</td>
</tr>
<tr>
<td>Hand washing and preoperative disinfection</td>
<td>Christensen + Ruston et al</td>
<td>22-23</td>
</tr>
<tr>
<td>Antibacterial effect on vaginal Bacteria</td>
<td>Vorherr H et. al.</td>
<td>31</td>
</tr>
<tr>
<td>Anti microbial effect on penis</td>
<td>Sanderson and Weissler</td>
<td>28</td>
</tr>
<tr>
<td>Oral efficacy</td>
<td>Miscellaneous</td>
<td>13,25, 60,61</td>
</tr>
<tr>
<td>Chlamydia trachomatis and common Bacterial S T D's</td>
<td>Temmerman M.</td>
<td>65</td>
</tr>
<tr>
<td>Staphylococcus faecalis, S. aureus, S. epidermidis</td>
<td>Christensen et. ak.</td>
<td>33</td>
</tr>
<tr>
<td>Group B Streptococci Long lasting microbial activity</td>
<td>Christensen et. al.</td>
<td>34, 35, 36, 37</td>
</tr>
<tr>
<td>Log reduction of 1.24 and 2.72. After six hours mean log 1.94 and 3.94 respectively</td>
<td>Easmon CS et. al.</td>
<td>36, 37</td>
</tr>
<tr>
<td>0.1% CH. - Log RF 1.62 after 3 minutes, and Log RF 3.25 after 30 minutes</td>
<td>Wewalka G. et. al.</td>
<td>66</td>
</tr>
<tr>
<td>Enveloped viruses; Herpes virus, Cytomegalovirus Influenza, Parainfluenza, Hepatitis B.</td>
<td>Bernstein D. et. Al</td>
<td>9</td>
</tr>
<tr>
<td>CHG proved to be effective as antiviral, within 1 min on HIV in the presence of whole human blood</td>
<td>Wood A, Payne D</td>
<td>74</td>
</tr>
<tr>
<td>Herpes Simplex Virus (HSV-1)</td>
<td>Park and Park</td>
<td>13</td>
</tr>
<tr>
<td>Rapid inactivation of infectious pathogens by CHG coated gloves</td>
<td>Modak S, et. al.</td>
<td>69</td>
</tr>
<tr>
<td>0.25% in the birth canal for 3 consecutive months on 3635 women giving birth.</td>
<td>Taha TE, et. al.,</td>
<td>73</td>
</tr>
<tr>
<td>Significant activity against HIV-1 in Semen and cervical secretions</td>
<td>O’Conor TJ et. al.</td>
<td>70</td>
</tr>
<tr>
<td>Inhibits the synthesis of viral DNA of host cell</td>
<td>Park NH et. al.</td>
<td>14</td>
</tr>
<tr>
<td>Human Immunodeficiency Virus (HIV)</td>
<td>Montefiory DC et. al.</td>
<td>15</td>
</tr>
<tr>
<td>Immediate inactivation of HIV</td>
<td>Haribson and Hammer</td>
<td>16</td>
</tr>
<tr>
<td>Total loss of sperm motility</td>
<td>Sharmar D. et. al.</td>
<td>17</td>
</tr>
<tr>
<td>Prevents migration through cervical mucus</td>
<td>Chijioke &amp; Chantler et al</td>
<td>18-19</td>
</tr>
<tr>
<td>Clinical research on Gel-x™</td>
<td>Bentwitz, Insler et al</td>
<td>68</td>
</tr>
<tr>
<td>Anti-HIV activity of Gel-X</td>
<td>Dr Robin J. Shattock</td>
<td>85</td>
</tr>
</tbody>
</table>
23. Martindale Extra Pharmacopoeia

The Martindale Extra Pharmacopoeia\(^{(67)}\) (30th Edition, page 789) of 1993, suggests the use of Chlorhexidine as a method to reduce the risk of transference of HIV, and states, "Chlorhexidine also has potential for reducing transmissions of HIV infection, as it does not disrupt the epithelium, and has activity in vitro against HIV virus in low concentrations."

Martindale\(^{(79)}\) page 786 defines that “BZC inactivates HIV in vitro. This property may contribute to the prevention of sexually transmitted disease when BZC is used as a spermicide. NX-9 has similar activity and in discussing that spermicide the view has been expressed that undetected low grade epithelial or mucosal damage arising from spermicide use may render the tissues more permeable to HIV”.

24. Patent

Patent Rights for Gel-x™ were granted in the European EEC countries, Australia, Canada and Israel but have since lapsed.

In the U.S.A, the application was pending for 18 years, and recently in May 2010, U.S. patent No. US 7,718,642 B2 valid through 11th August 2016 was granted.

The patent covers and defends all products that fall under the following category:

A prophylactic lubricant composition for use during sexual relations, comprising:

a) a lubricant effective to reduce friction;

b) an effective amount of spermicidal antiseptic, active against STD's, which immobilizes the sperm and prevents its penetration into the uterus;

c) an effective amount of fungicide to prevent the growth of fungi during the absence of natural flora;

said composition having no substantial detrimental side effects.

The patent, as can be seen from the above, covers any vaginal microbicide which has the properties of vaginal lubricant + STD destruction + contraceptive + effective fungicide. This composition is the minimum construction, which can be approved for vaginal use and its efficacy, and safety was demonstrated in the above article.

All above suggested ingredients for Gel-x™ are already used in the vagina, which gives them an advantage over ingredients, which are not currently used in the vagina. New ingredients require years of testing. On the other hand, as the ingredients suggested by us are already known, without foresight it would not have been possible to obtain intangible protection rights. When the product becomes successful, it will be copied immediately after medical registration, as in a generic product. The patent, as described above, will avoid this problem.
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