

PRE-OPERATIVE DEGERMING
OF THE HANDS

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By
GOVIND C. THAKKER
M.S., B.A., D.L.D.

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By
GOVIND C. THAKKER
M. B., B. S., D. L & O.

PRE-OPERATIVE DETERMINING OF THE HANDS

DISSERTATION

FOR

THE DEGREE OF

MASTER OF SURGERY

(PART I)

BY

Dr. Govind C. Thakker

MEDICAL COLLEGE AND SHREE SAVALI GENERAL HOSPITAL

B A R O D A

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-: A C K N O W L E D G E M E N T :-

I express my sincere thanks to the Dean, Medical College and Superintendent, S.S.G. Hospital, Baroda for his kind permission to use hospital materials.

I wish to express my indebtedness to Dr. J.G. Collee, M.D. (Edin), W.H.O. Visiting professor of bacteriology, who encouraged me for this work and for his valuable guidance. Without him this work would not have been possible.

I am sincerely thankful to my teacher Dr. A.B. Kothari, M.S., F.R.C.S. (G), F.I.C.A., F.A.C.S. Hon. Surgeon and Post-Graduate teacher in Surgery for his valuable guidance given to me.

I also thank Dr. M.A. Patel, M.D., F.R.C.S., for his valuable and timely guidance. I also thank Dr. R.C. Desai, Dr. M.S. Patel for the encouragement given to me.

I am grateful to Dr. B.A. Sayed, Dr. D'Souza, Dr. Balar and people of the bacteriology department, Medical College, Baroda, for their help.

I am indebted to Dr. T.J. Roman, M.Sc., M.S., Ph.D. Officer-in-charge (Class-I), State public health laboratory, Baroda, He allowed me to use his laboratory steriliser to sterilize all the material.

I also thank all the volunteers who kindly co-operated in this work.

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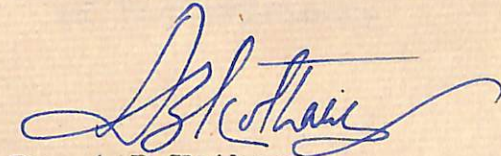
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C E R T I F I C A T E

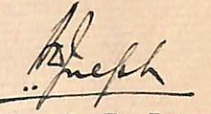
This is to certify that the enclosed work on "Pre-Operative Degerming of The Hands", has been done by Dr. G.C. Thakker, M.B.B.S., D.L.O., at the S.S.G. Hospital and Medical College, Baroda, under the guidance and supervision of Dr. A.B. Kothari, M.B., M.S. (Madras), F.R.C.S. (G), F.I.C.S., F.I.C.A., F.A.C.S., the Honorary Surgeon and Post-graduate Teacher, S.S.G. Hospital and Medical College, Baroda.

Baroda :

Dated : 15/12/64



Dr. A.B. Kothari
M.B., M.S. (Madras),
F.R.C.S. (G),
F.I.C.S., F.I.C.A., F.A.C.S.,
Honorary Surgeon and
Post-graduate Teacher,
S.S.G. Hospital & Medical College,
BARODA.



Dr. A.D. Joseph,
M.D., F.C.P.S.,
Dean
Medical College &
Superintendent
S.S.G. Hospital,
BARODA.

5th June 1964.

I hereby certify that
 Dr. G.C.Thakker has worked under my
 guidance on his investigations into
 degerming of hands. I certify that
 he has performed the work personally
 and industriously.

He has my best wishes.

J.G. Collee
 Dr. J.G.Collee
 M.D., M.C.Path.,
 W.H.O. Visiting Professor
 of Bacteriology.

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1931 June 15th

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guidance on his investigation into
the nature of the disease I hereby
certify that he has performed the work personally
and industriously.

He has my best wishes.


W.H.G. Vesting
M.D., D.V.M.
Professor
of Bacteriology

INTRODUCTION:-

At present it is not possible to sterilize the skin completely without damaging the tissues. For this reason "Degerming of the skin" is the phrase which is now being used to describe pre-operative removal of bacteria from the skin, and it is used in preference to "sterilization of the skin".

The skin may harbour many bacteria and although certain species are common, the flora varies from person to person. There is no known relation with age, sex, or race of a person. Some are heavy carriers while others harbour small number of organisms.

Skin flora can be divided into two groups:

(a) Transient flora - which may be easily removed by simple washing and are found in superficial layers of the skin. These organisms are acquired by contamination from various articles in daily use.

(b) Resident flora - They are difficult to get rid of. They withstand ordinary washing and are perhaps situated in the ducts of sweat and sebaceous glands.

A large amount of work has been done on this problem in many countries. There is still no one method which is reliable for the removal of the resident flora.

The surgeon may employ any one of the following procedures to remove bacteria from his hands pre-operatively:

1. Ordinary cake soap application and brushing (Time factor varies upto 10 minutes.)

2. Liquid soap used in similar manner.

3. 1 or 2 followed by alcohol rinse or lint friction with alcohol.

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4. 1 or 2 followed by rinse with 70% alcohol containing 0.5% chlorehexidine.

5. Cake or liquid soap containing "G-11" (Hexachlorophene) for 3 minutes.

6. Other preparations may be incorporated in similar procedures, e.g. use of "Tegolan" as a substitute for soap.

Recently in European countries and America soap containing Hexachlorophene has come into common use.

HisoHex is phisoderm, a liquid detergent containing 3% hexachlorophene.

In this hospital there are 8 operation theatres where many surgeons and resident staff are working. There is no standard scrubbing technique.

Water supply is from the head tanks at atmospheric temperature. In winter days, especially in the morning, water is so cool that sub-consciously the scrubbing time is cut-short.

Some of the taps have single outlet while others are of the shower type. All have long handles which may be operated with the elbow.

When main water supply is not available, water is kept in a reservoir tank which has a cock which cannot be operated by the same person during washing. When this fails, a second person pours water from a jug, on to the hands.

The wash-basins available vary greatly in size. Spillage from small basins splashes on the body and the floor. The level of the taps is rather low.

The soap used for washing is not standardized. Many proprietary preparations, like SUNLIGHT, LIFEBOUY, HAMAM and

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on some occasions hard soap; are being used.

Time factor also varies from person to person and from occasion to occasion. In three theatres sand operated hour glasses (5 minutes) are provided on wash basins so that proper timing is ensured. In one theatre an electrically operated clock guides the surgeons during washings.

Observed times of pre-operative wash varied from 1/2 minute to 4 minutes.

The nail brush is used by very few people. Nail brushes are kept in an open bowl containing water of doubtful sterility. It is said to be sterilized only once in the morning. During the whole session the same brush is repeatedly used from the same bowl containing soapy water (due to previous washings) giving the impression of some antiseptic solution.

After washing hands, some operators dry their hands with sterile wipers, while others utilise a glove bag or rub their hands on the gown which they are wearing.

After this powder is sprinkled on the hands and autoclaved gloves are put on.

In the recent past some of the gloves were boiled and kept in a bowl. These were used wet after lubricating hands with ether soap.

In this project an attempt is made to study the deficiencies and advantages of some of the techniques employed. Attention has also been paid to sources of transient contamination of the skin of the surgeon and his patient.

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HISTORICAL REVIEW :

Until the present century sepsis was very common (rather healing by 1st intention was exceptional) in surgical wounds. The work of Pasteur and Lister revolutionized the practice of surgery and improvements began 100 years ago.

In 1861 Lister began to teach in Glasgow that the occurrence of suppuration in wounds is a result of decomposition. He knew the secret of it in 1815 from the writings of Pasteur that putrefaction was due to air borne organisms.

He introduced carbolic acid in surgery. He thought that he should deal with germs present in the wounds, on HIS HANDS and in the air.

Ignaz Philipp Semmelweis (1818-85) was assistant in the obstetric unit at general hospital at Vienna. The death rate in puerperial patients ranged between 10-30% of the pregnant women admitted. Deliveries were conducted by students who came from pathology lectures, dissection room, or post-mortem rooms. "By insisting on sterilization of the hands of the operators Semmelweis succeeded in 1846 in reducing mortality at once to 1% of the pregnant women admitted.

His methods aroused great opposition and his work was soon forgotten.

In olden days surgeons used to put on special coat while operating. It was not even kept clean.

In 1880 white gown was introduced and surgery started entering ASEPTIC AGE from antiseptic era.

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antiseptic lotions. They had much faith in those magic solutions.

Quoting Dr. Clavin M. Smith - "The surgeon could be recognized in the public by the fact that his nails were deeply stained with bichloride".

In 1890 W. Halsted introduced rubber gloves which are now universally used during operations.

The story behind the introduction of surgical gloves runs like this : -

"A few years before, the head nurse of the Hopkin's operating room had complained that the harsh antiseptics were making her hands painfully sore, and taking an idea from the heavy coach man's gloves Dr. Welch used for performing autopsies, Halsted asked a New York manufacturer to make her a pair of thin rubber gloves. They served her so well that assistants took to wearing them too", and thus were introduced the surgical gloves.

Past generation of surgeons was very particular about scrubbing. With the advent of sulpha drugs and antibiotics people have become negligent about aseptic precautions and amongst them hand scrubbing is neglected to much extent.

There is a school of thought which holds that modern surgeons and particularly younger ones are more careless of the aseptic precautions than were the surgeons in the days before antibiotics.

The surgical scrub with soap and brush is a notoriously tedious process, hence many people have sought a solution to this problem.

Various substances have been found to reduce the number of bacteria on the hands. Many antiseptics have received

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enthusiastic acceptance only to be discarded later; not because they failed in clinical trials but because improved methods of testing showed them to be less effective bacteriologically than had been supposed.

An ideal material for scrubbing should possess the following characteristics:- (S.M.Joress)

1. It should be effective against resident as well as transient bacteria.
2. It should kill or remove all types of Micro-organisms.
3. Application should be quick and its effect should be sustained.
4. It should be used any where without irritation or sensitivity.
5. It should not be rendered ineffective by Common material like alcohol, serum, organic matters soap etc.
6. (It should be easily available and cheap.)

Amongst the antiseptics used to disinfect the skin alcohol is used from long time.

Before few years mercuric iodide was said to be a potent antiseptic and hands were dipped in a solution of mercuric iodide after surgical scrub. It is a salt of heavy metal which acts by precipitating the proteins and inhibiting the sulph-hydryl group in enzyme system which is so essential to bacterial growth. It is an irritant hence cannot be used on abraded skin.

Skin irritation is known with mercuric salts in some persons.

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"Cetavlon (cetrimide B.P.) tetra-decyl alcohol - Mixture of dodecyl, tetradecyl, and hexadecyl trimethyl ammonium bromides, was introduced in medicine in 1942. Since that time its quality has been gradually improved as a result of continuous research, and is one of the most popular antiseptics of to-day.

Hibitane (Chlorhexidine) is an antiseptic of an entirely new chemical type synthesised and investigated in the laboratories of I.C.I. by Davies et al 1954; and Rose and Swain 1956."

Dettol is another popular antiseptic commonly used. The bacterial activity of DETTOL is based on parachlore metaxyleneol and TERPINEOL.

Lastly came HEXACHLOROPHENE or commonly called G-11, which has become very popular in western countries and United States.

Pioneer work is that of Dr. Philip B. Price who introduced scientific study of various substances used for scrubbing. In thirties he introduced "serial wash basin technique" which is commonly used.

In 1944 Traub, Newhall and Fuller showed a marked decrease in skin flora following the daily use of soap containing G-11.

In 1947 Seastone published the result of his experimental work showing the beneficial effect of hexachlorophene as a skin antiseptic. This was followed by the work of Hufnagal, Walter and Holland who in 1948 combined 3% G - 11 with pHisoderm.

pHisoderm is a soapless water miscible anionic detergent cream. It contains a sulphonated ether, wool fat, cholesterol and petroleum and is a surface tension reducent 40% more powerful than soap.

In 1951 Dr. Philip B. Price delivered a lecture before

The American Medical Association and stressed the limitations of G-11 soap. He stressed that when hexachlorophene is used for surgical scrub the immediate effects are not superior to ordinary bar soap.

"It is necessary to use G-11 soap exclusively and frequently" to decrease the bacterial skin flora and the effect is attributed to a film of the agent left on the hands. It does not disinfect as quickly as alcohol.

In 1955 Murray and Calman showed that the use of the CHLORHEXIDINE cream in obstetric practice decreased the incidence of cross infection.

This was followed by the works of Smylie, Webster and Bruce; E.L.J. Lowbury and H.A. Lilly; Ralph C. Richards etc.

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MATERIALS AND METHODS :-

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In this work some of the pre-operative scrubbing techniques were studied to know their advantages and deficiencies.

Various pre-operative preparations of the patients skin were not included in this study.

In the first place swabs were taken from the anterior nares, back of the fore-arms, cubital fossae of the volunteers to find out how many of them were carriers of S.aureus. In 11 out of 12 volunteers I could do the perineal swab examination for carriage of S.aureus.

The nasal swabs were prepared on a thin sticks with cotton just enough to cover the stick end, and were autoclaved at 15 lbs. pressure for 20 minutes. Control studies showed complete sterility.

The nasal swabs were collected from the volunteers as follows: Volunteer was asked to raised the chin a little and not to breathe in or out during the procedure. The swab was introduced for about 1/2" in the external nares. The material was collected by 6 circumferential clock-wise turn in the left nostril and anti-clock wise turns in the right nostril.

The material was inoculated on 10 cms. diameter nutrient agar plates as early as possible (within about 20 minutes). 10 complete strokes were made in 1/2 plate, then plate rotated to 60° Now 5 strokes made and lastly tailing.

The plates were incubated at 37° C for 24 hours and then kept in light at room temperature for the pigmentation to develop. Colony count was recorded as usual.

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Golden yellow looking colonies were selected; part of the colony picked up and slide co-agulase test performed. Remaining part inoculated on the agar slope. The result of the slide test noted. Next day the colony picked up from the agar slope and tube co-agulase test performed and the result noted.

Doubtful colonies, and in absence of golden yellow looking colonies white colonies, were tested for co-agulase activity. Those organisms which were found co-agulase positive were tested for their antibiotic sensitivity and result noted.

In the next investigation swabs were taken from both the cubital fossae and the dorsum of each fore arm. One swab was rubbed on both cubital fossae (10 complete strokes on either side with the swab rotating) Two swabs were used for two fore-arms (10 complete strokes) The swabs were inoculated as usual. Lastly perineal swabs were collected and investigated as usual.

(Volunteers were instructed that swab should be rubbed on the perineum avoiding the anal region.)

After this preliminary survey different scrubbing techniques were investigated.

The methods were standardized as much as possible.

For each sample 100 mls. of nutrient broth was used and was kept in two 50 mls. screw capped bottles and autoclaves.

Carefully cleaned stainless steel bowls (30 cms. diameter, 10 cms. depth.) were wrapped in clean towels, autoclaved and then used promptly for the hand washings.

The technique of sample collection was as follows:

Materials: A. One autoclaved stainless steel bowl wrapped in towel.



- B. Two 50 mls. screw capped bottles containing 100 mls. nutrient broth.
- C. Two 10 mls. screw capped bottles containing 9 mls. (each) nutrient broth, and one 1 ml. screw capped bottle.
- D. 1 ml. pipettes in a copper container (sterilized)
- E. One autoclaved nail-brush.
- F. Soap etc.

Method - (Washing time for a "Social Wash" was a controversial point and it was decided that 15 seconds soap application and 15 seconds wash in running tap water is a good time for a social wash.

Alexander J. Gordon considered 45 seconds washing time for a "Social wash").

METHOD OF COLLECTION OF SAMPLE:-

The volunteer was asked to rub palm to palm 10 times and 25 ml. of the nutrient broth was poured from one of the 50 mls. bottles, and the washings collected in a bowl. Then he rubbed the dorsum of the left hand with the palm of the right hand with the fingers interlocking, 10 times. Again remaining 25 mls. nutrient broth poured on the hands during the procedure and the washing collected in the same bowl. The procedure was repeated with the hands changed. Then the finger tips of the right hand were rubbed on the left palm 10 times. During the procedure 12.5 (approx.) mls. of the nutrient broth poured and the washing collected in the bowl. Once again the procedure was repeated with the hands changed.

In this way 100 mls. of the washing was obtained in the bowl which was then covered and kept aside.

A. 1st technique consisted of washing hands with SUNLIGHT soap in running tap water. Before starting washing it was seen that nails were short.

1. Initial hand rinse was sampled prior to washing.

Golden yellow looking colonies were selected; part of the colony picked up and slide co-cultured test performed. Remaining part inoculated on the agar slope. The result of the slide test noted. Next day the colony picked up from the agar slope and tube co-cultured test performed and the result noted.

Hemolitic colonies, and in absence of golden yellow looking colonies white colonies, were tested for co-cultured activity. Those organisms which were found co-cultured positive were tested for their antibiotic sensitivity and result noted.

In the next investigation swabs were taken from both the cubital fossa, and the dorsum of each fore arm. One swab was rubbed on both cubital fossa (to complete strokes on either side with the swab rotating). Two swabs were used for the fore-arms (10 complete strokes). The swabs were inoculated as usual. Lastly, perineal swabs were collected and investigated as usual.

(Volunteers were instructed that swab should be rubbed on the perineum avoiding the anal region.)

After this preliminary survey different scrubbing techniques were investigated.

The methods were standardized as much as possible.

For each sample 100 mls. of nutrient broth was used and was kept in two 50 mls. screw capped bottles and autoclaved.

Carefully & evenly stainless steel bowls (30cm. diameter, 10 cm. depth.) were wrapped in clean towels, autoclaved and then used promptly for the hand washings.

The technique of sample collection was as follows:

Materials: A. One autoclaved stainless steel bowl wrapped

Then washing was commenced. The details of the technique are as follows:-

2. Wet the hands (only palm and dorsum upto wrist).
3. Sunlight soap was lathered and applied for 15 seconds.
4. Hands washed in running tap water for 15 seconds.
5. Second sample collected.
6. Wet the fore arms upto elbows.
7. Sunlight soap was lathered and applied for 30 seconds.
8. Hands washed in running tap water for 15 seconds with water running from wrist to elbow.
9. Sunlight soap lathered and applied for 15 seconds.
10. Brushing the finger tips, with special attention to nails, with a sterile nail brush for 15 seconds (about 10 complete strokes on each hands).
11. The hands washed in running tap water for 30 seconds.
12. Sunlight soap lathered and applied ^{for} 30 seconds.
13. Hands washed in running tap water for 15 seconds.
14. Sun-light soap lathered and applied for 30 seconds.
15. Hands washed in running tap water for 15 seconds.
16. Sunlight soap lathered and applied for 30 seconds.
17. Hands washed in running tap water for 45 seconds.
18. Third sample collected.

The bowls were taken, shaken, pipette fitted with the rubber cap and rinsed with the solution. 2.5 - 3.0 mls. of the washing collected in an autoclaved screw capped 5 ml. bottle ~~containing 2 mls~~ and label applied. (This was undiluted sample.) In another 10 mls. bottle containing 9 ml. of nutrient broth, 1 ml. of the washing added, giving 1:10 dilution, and labelled accordingly. Similarly all samples were collected in the sample bottles and kept in the refrigerator till they were inoculated on the media.

1. Two 50 ml. screw capped bottles containing 100 mls. nutrient broth.
2. Two 10 ml. screw capped bottles containing 9 mls. (each) nutrient broth and one 1 ml. screw capped bottle.
3. 1 ml. pipettes in a copper container (sterilized).
4. The autoclaved nail-brush.
5. Soap etc.

Washing time for a "social wash" was a controversial point and it was decided that 15 seconds soap application and 15 seconds wash in running tap water is a good time for a social wash.

Alexander's method considered 15 seconds washing time for a "social wash".

METHOD OF COLLECTION OF SAMPLES:-

The volunteer was asked to rub palm to palm 10 times and 20 ml. of the nutrient broth was poured from one of the 50 ml. bottles, and the washings collected in a bowl. Then he rubbed the dorsum of the left hand with the palm of the right hand with the fingers interlocking, 10 times. Again remaining 25 mls. nutrient broth poured on the hands during the procedure and the washing collected in the same bowl. The procedure was repeated with the hands changed. Then the finger tips of the right hand were rubbed on the left palm 10 times. During the procedure 10 mls. of the nutrient broth poured and the washing collected in the bowl. Once again the procedure was repeated with the hands changed.

In this way 100 mls. of the washing was obtained in the bowl which was then covered and kept aside.

The technique consisted of washing hands with Sunlight soap in running tap water, before starting washing. It was seen that nails were short.

1. Initial hand time was sampled prior to washing.

10 cms. diameter nutrient agar plates selected for each sample and dried in the incubator at 37°C for two hours.

Propagating pipettes (50 drops/ml.) were prepared from the glass tubes and kept ready.

Each petri-dish was marked with a glass pencil on the back and divided in two sectors and labelled accordingly (one for undiluted sample and other for 1:10 dilution).

Water was kept boiling and by the side in which pipette could be sterilised, and cool sterile water to bring down the temp. of the pipette before it was introduced in the sample bottle.

The sample bottle was shaken, the cap opened and pipette rinsed with the sample fluid by sucking in and forcing out several times. This serves two purposes....the material in the bottle will be shaken and the fluid in the pipette will be of uniform concentration as in the container.

A little time was allowed for the mixture to settle so that the volume of the drop was not influenced by the air bubbles.

Some fluid was taken in the dropping pipette. Few drops were discarded to get uniform drops.

The dish was opened and 5 drops were inoculated in the allotted sector. Inoculation was started from the higher dilution to full concentration. Similarly plates for all samples were inoculated and kept on the side with the lid on, till the fluid was absorbed by the medium and then incubated at 37°C.

After 18-20 hours of incubation plates were taken out of the incubator and the colony count was done. (See photographs)

The number of bacteria 100 mic. of undiluted fluid was calculated and the result noted.

Pre-scrub sample inoculated on the agar plate.

Sample after social wash inoculated on the agar plate.

Post-scrub sample inoculated on the agar plate.

Plate showing antibiotic sensitivity of S. aureus

Theatre nail brush Impression showing profuse growth of coliform organisms.

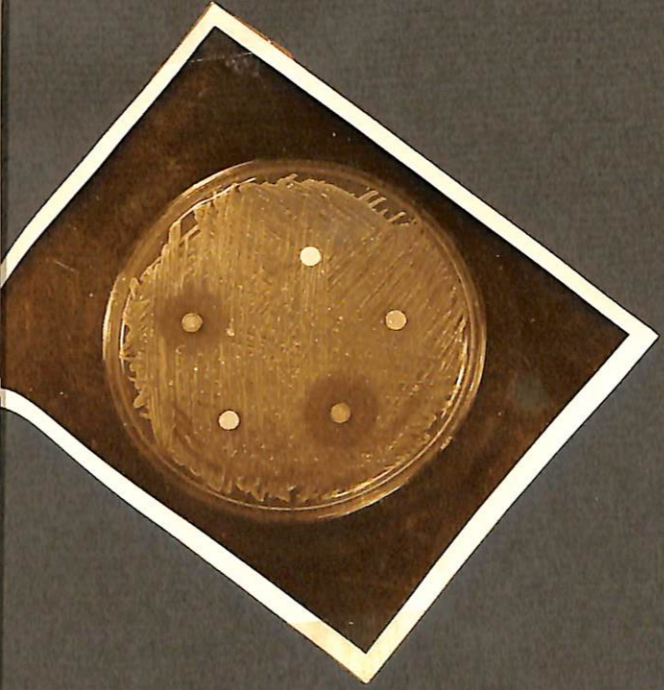
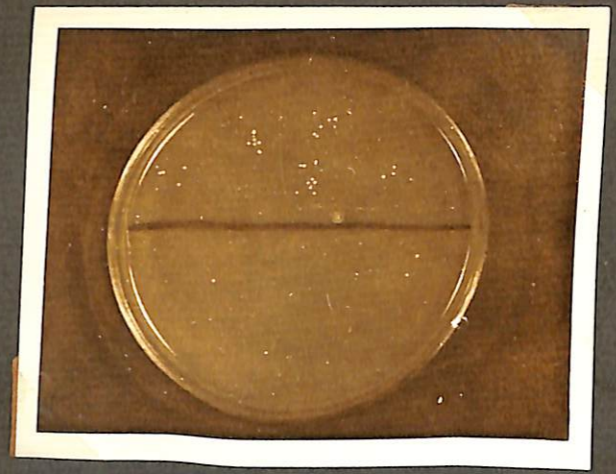
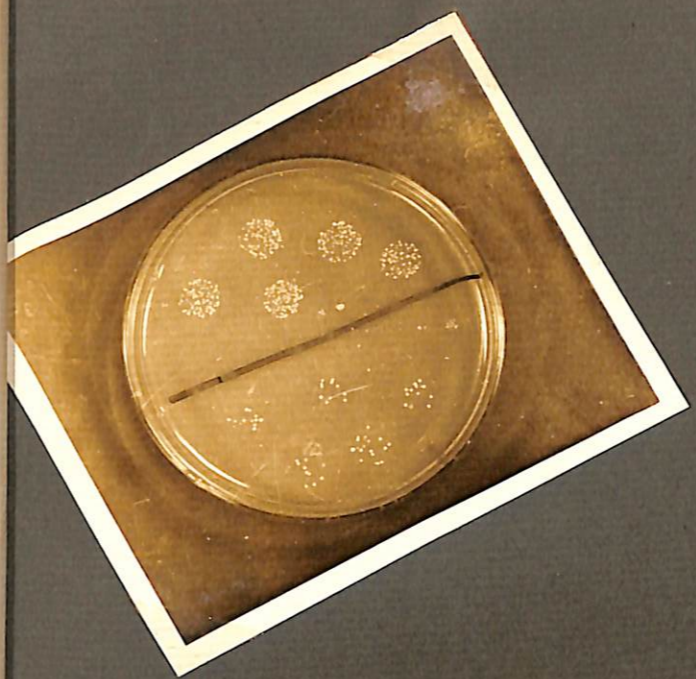
Sample after social
wash inoculated on
the agar plate.

Pre-scrub sample
inoculated on the
Agar plate.

Post-scrub sample
inoculated on the
Agar plate.

Theatre nail brush
Inhibition showing
Profuse growth of
coliform organisms.

Plate showing antibiotic
sensitivity of *S. aureus*



B. 2nd technique is like first technique till collection of 3rd sample.

After that the hands were washed in running tap water for a short time to remove the sampling fluid.

Then 10 ml. of absolute ethyl alcohol was poured on the hands and volunteer asked to rub the hands in a standard way. (palm to palm 10 times, dorsum of left hand with palmar surface of right palm with fingers inter-locking, similarly with the other hand, that is hands were reversed, and lastly the finger tips of each hand on the palmar surface of the other hand for 15 seconds.) This was allowed to act for 2 minutes.

The hands were kept in such a position that no water was allowed to flow on the palm from the fore-arms.

Then the hands were washed in running tap water for 45 seconds to remove the remaining trace of alcohol lest it may interfere with the sampling fluid; and forth sample collected.

C. In the third technique the effect of ETHER soap was studied. It is prepared in the hospital dispensary.

Composition:- Saponis mollis 4 parts.
Ether 3 parts.
Methylated
spirit 3 parts.
Distilled
water 20 parts.

The basic pattern of the procedure was same as in the first technique - instead of sunlight soap ETHER soap, after thorough shaking the stock bottle, 5 mls. each was kept in the sterile plain 5 mls. stoppered bottles, was used.

After the first sample was collected the hands were washed to wash out the solution. Then 5 mls. of ETHER soap was

poured and rubbed all over the hands, distal to the wrists for 15 seconds.

Then it was washed for 15 seconds under running tap water and second sample collected.

Solution washed out. Later hands were scrubbed up to elbows as in the first technique. Instead of SUNLIGHT SOAP 15 mls. of ETHER soap was used each time. (5 mls. for palms, 5 mls. for right fore-arm, and 5 mls. for the left fore-arm.) In all 80 mls. of ETHER soap was used for each volunteer for each scrub up.

After 5 minutes scrub third sample was collected.

The samples were taken to Bacteriology laboratory and investigated as usual.

D. In the next technique "NEKO" soap (Parke Davis) was considered. It contains as its active ingredient Mercuric Iodide 1% W/W (N.F.).

IT WAS FOUND IN THE CONTROL STUDIES THAT THE EFFECT OF MERCURIC IODIDE REMAINED ON THE HANDS EVEN AFTER WASHING THEM IN RUNNING TAP WATER FOR 45 SECONDS AND THIS "CARRIED OVER" EFFECT GAVE FALSE NEGATIVE RESULTS; HENCE ALONG WITH THE NUTRIENT BROTH SODIUM THIO-SULPHATE 2% WAS UTILISED TO COUNTERACT THE EFFECT OF MERCURIC IODIDE CARRIED OVER.

Addition of Sodium thio-sulphate to nutrient broth did not interfere with the growth of the bacteria on the agar plates.

H. 2nd technique is the first technique till collection of 3rd sample.

After that the hands were washed in running tap water for a short time to remove the sampling fluid.

Then 10 ml. of absolute ethyl alcohol was poured on the hands and volunteer asked to rub the hands in a standard way. (palm to palm 10 times, dorsum of left hand with palmar surface of right palm with fingers inter-locked, similarly with the other hand, that is hands were reversed, and finally the tips of each hand on the palmar surface of the other hand for 15 seconds.) This was allowed to act for 2 minutes.

The hands were kept in such a position that no water was allowed to flow on the palm from the fore-arms.

Then the hands were washed in running tap water for 45 seconds to remove the remaining trace of alcohol. Last 10 ml. of water was used for the sampling fluid; and third sample collected.

D. In the third technique the effect of 1% Iodine soap was studied. It is prepared in the hospital dispensary.

Composition:-

Saponis mollis 4 parts.
Ether 2 parts.
Methylated
spirit 3 parts.
Distilled
water 20 parts.

The basic pattern of the procedure was same as in the first technique - instead of sunlight soap 1% Iodine soap, after thorough shaking the stock bottle, 5 ml. each was kept in the sterile 5 ml. stoppered bottles.

After the first sample was collected the hands were washed to wash out the solution. Then 5 ml. of ETHER soap was

In these technique 5 mls.of 20% Sodium thiosulphate was added to each 45 mls.of the nutrient broth and kept in the autoclaved bottles.This was done before starting the experiment in order to counteract the "CARRIED OVER" effect of Mercuric iodide.

The procedure was same as in the first technique,instead of Sunlight soap NEKO soap was used,and samples collected according to the first technique,and examined in Bacteriology laboratory.

E. In this technique effect of DETTOL rinse after 5 minutes Sunlight soap was studied, and pre scrub and at the end of 5 minutes Sunlight soap scrub the sampjes were collected as usual.

After 5 minutes scrub the hands were treated as in "Spirit"rinse with DETTOL 5 mls.and samples collected.

On washing hands under running tap water it was^{found} that DETTOL was completely removed and there was no "carried over"effect

Then it was washed for 15 seconds under running tap water and second sample collected.

Solution washed out, later hands were scrubbed up to elbows as in the first technique, instead of SUNLIGHT SOAP 15 mls. of ETHER soap was used each time. (5 mls. for palms, 5 mls. for right fore-arm, and 5 mls. for the left fore-arm.) In all 80 mls. of ETHER soap was used for each volunteer for each scrub up.

After 5 minutes scrub third sample was collected. The samples were taken to Bacteriology laboratory and investigated as usual.

D. In the next technique "NEKO" soap (Parke Davis) was considered. It contains as its active ingredient Mercuric Iodide (0.1%).

IT WAS FOUND IN THE CONTROL STUDIES THAT THE EFFECT OF MERCURIC IODINE REMAINED ON THE HANDS EVEN AFTER WASHING THEM IN RUNNING TAP WATER FOR 45 SECONDS AND THIS "CARRIED OVER" EFFECT GAVE FALSE NEGATIVE RESULTS; HENCE ALONG WITH THE NUTRIENT BROTH SODIUM THIO-SULPHATE 2% WAS UTILISED TO COUNTERACT THE EFFECT OF MERCURIC IODINE CARRIED OVER.

Addition of Sodium thio-sulphate to nutrient broth did not interfere with the growth of the bacteria on the agar plates.

RESULTS:-

Totally 12 volunteers were selected and all were bacteriologically screened.

Swabs were taken from the anterior nares, back of right and left fore arms, cubital fossae and perineum. The result is represented in a tabular form. Nasal swabs of all the volunteers showed heavy growth mainly of *S. albus*. *Staphylococcus albus* is a commensal in the anterior nares and was found in all volunteers in good numbers.

Of the 12 volunteers 4 were found to be carriers of *Staphylococcus aureus*. (coagulase test positive).

Back of the arms showed non-pathogenic *Staphylococci* (*S. albus* and *S. citreus*). Number of colonies per unit area on the back of the fore arms were much less compared to the anterior nares which harbour enormous number of bacteria.

Swabs from the cubital fossae revealed the presence of *S. albus*, *S. citreus*, Micrococci, and occasionally *S. aureus*.

Perineal swabs revealed profuse growth of the coliform organisms and micrococci. In majority of the swabs (except the nasal and the perineal swabs) the number of colonies were below 50 per plate.

One volunteer revealed only 3 colonies in cubital fossa swab.

S. albus - 1 colony
**S. aureus* - 1 colony (Coagulase test positive)
Coliform - 1 colony

It was difficult to get perineal swabs, some volunteers were hesitant to give, while one volunteer refused to give the perineal swab. No volunteer was harbouring *S. aureus* in the perineum.

CHART SHOWING ANTIBIOTIC SENSITIVITY OF S. AUREUS (Cultured from the Volunteers)

(By disk diffusion technique)

Volunteer Number	Nasal Swab	Cubital Fossae Swab	Penicillin (10 unit/disk)	Streptomycin (10 µgm/disk)	Erythromycin (10 µgm/disk)	Tetracycline (10 µgm/disk)	Chloramphenicol (25 µgm/disk)
1	"	-	R.R.	S.	S.	S.	S.
2	"	-	R.	R.	S.	R.	S.
4	"	-	R.	R.	R.R.	R.R.	S.
10	"	-	R.	R.R.	R.R.	S.	S.
7	-	"	R.R.	R.R.	S.	S.	S.

S. -- Sensitive; R.R. -- Relatively Resistant; R. -- Resistant.

1 ml. of the tap water was collected in a sterilized screw capped bottle and inoculated on the nutrient agar plate. After 24 hours incubation at 37°C not a single colony was found proving that water was sterile for all practical purposes.

1 ml. of the tank water was inoculated and incubated at 37°C for 20 hours -- No growth.

1 ml. of the water from jug was inoculated and incubated at 37°C for 20 hours -- Profuse growth of anthracoid organisms.

Swabs were taken from the wash basins and tap handles of Operation theatre number 1 and 2.

O.T. 1 wash basin	76 colonies	(S. albus)
O.T. 2 wash basin	27 colonies	(S. albus 26 colonies S. citreus 1 colony)
O.T. 1 tap handle	20 colonies	(S. albus)
O.T. 2 Tap handle	1 colony	(S. albus)
<u>No coliform organisms or S. aureus detected.</u>		

The impression of the nail brush from O.T.1 was taken directly on nutrient agar plate and incubated at 37°C. It was for the chief surgeon who was to perform Porto-caval shunt on that day. It should profuse growth of coliform organisms (see photograph).

Nasal swabs from 6 nurses were inoculated. The result was as in the previous volunteers, showing many colonies of S. albus and occasionally S. citreus. One nurse was harbouring plenty of S. aureus - only one colony was of S. albus; and the organisms were resistant to Penicillin & Streptomycin, relatively resistant to erythromycin & terramycin & sensitive to chloramphenicol only.

First technique consisted of washing hands with SUNLIGHT soap. Initial hand rinse was sampled prior to washing (Sample "A") After this hands were washed with Sunlight soap for 15 seconds and wash under running tap water for 15 seconds and the second sample was collected (sample "B") --- SOCIAL RINSE.

The surgical scrub-up consisted of a further 4½ minutes wash with Sunlight soap at the end of which a third sample was collected (sample "C").

In the first technique 10 volunteers were available. The count varied a lot, --- from 3,500,000/100 mls. to 70,000/100 mls.

After social wash the count varied from 8% to 142% of the original count.

After surgical scrub for 5 minutes the residue was 6% to 73% of the original count.

The samples were cultured on the agar plates by the MILES AND MISRA method. The (drop) area could be easily demarcated hence surrounding area could be checked as a control for contamination of agar plates which occasionally occurred.

Second technique consisted of surgical scrub with Sunlight soap for 5 minutes followed by spirit rinse (10 mls. of absolute alcohol rubbed for 30 seconds and allowed to evaporate which takes about 1½ - 2 minutes and washed for 45 seconds at the end of 2 minutes.

The actual count varied from 0-400,000 per 100 mls. of washing fluid.

Percentage of original count varied from 0-23% (see table 1 and 2)

In one of the plates there was contamination with coliform

CHART SHOWING ANTIBIOTIC RESISTIVITY OF

()

Volunteer Number

Hand	Sample	Count
Right	"	1
"	"	2
"	"	4
"	"	10
"	"	7

2. ---

1 ml. of the liquid was collected in a sterile agar plate. After 24 hours incubation at 37°C and a single practical purpose.

1 ml. of the liquid was inoculated and then

1 ml. of the culture was inoculated and the

- 0.1. 1 wash basin
- 0.1. 2 wash basin
- 0.1. 1 tap handle
- 0.1. 2 tap handle

to collect

The impaction of the ball from 0.1. 1. and

Small areas from 7 nurses were inoculated. The

organisms and counting was not possible, hence result not charted.

One volunteer had burning on the dorsum of one hand after spirit rinse.

In the third technique ETHER soap was tried. 7 volunteers were available for the test.

The residual count after social wash varied ~~wk~~ from 11,000-840,000/100 ml. 23.98% to 66.67% of the original count.

After surgical scrub for 5 minutes the count varied from 1,000-270,000/100ml. There was one contaminated plate.

Residual count after surgical scrub varied from 0.4696-46.56% of the original count, Nobody had any reaction on the skin.

In the fourth technique DETTOL was studied.

9 volunteers were studied in this technique. 2 samples were collected like First and Third samples of First technique.

Initial count was high in all volunteers (except in volunteer no. 10) probably due to summer and excessive perspiration and varied from 470,000 - 25,600,000/100 mls.

1. Percentage of residue after surgical scrub varied from 4.894-86.12% of the original count.

In the fourth technique which consisted rubbing 5 mls. of pure DETTOL in a standard way, after washing hands for 5 minutes with Sunlight soap, for 30 seconds and allowing it to act for 2 minutes before washing.

The count varied from 4,500-1,275,000/100 mls. and the residual count varied from 0.95 to 10.35% of the original count.

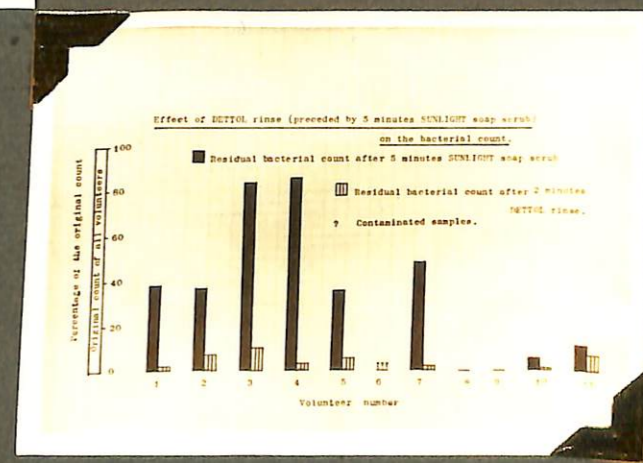
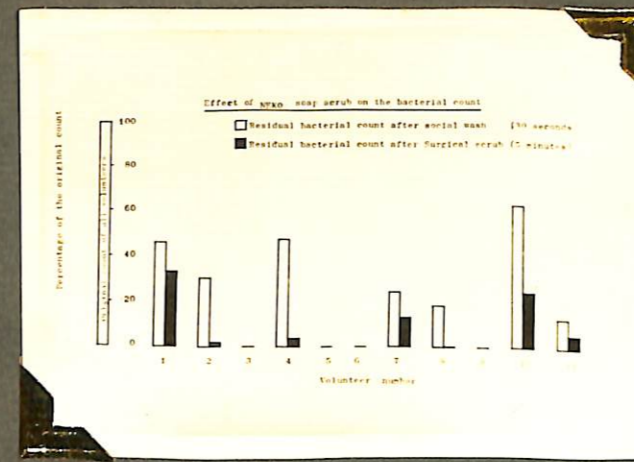
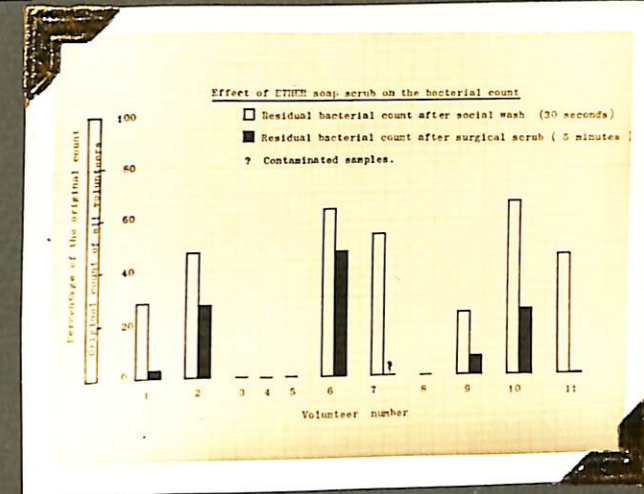
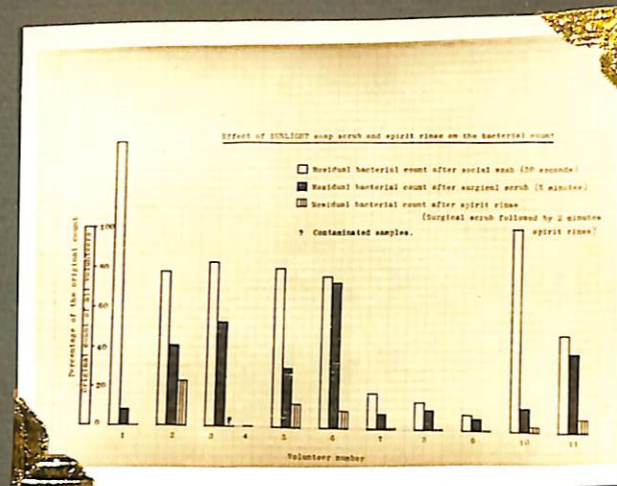
Almost all volunteers had burning on the hands, specially on the hairy areas, which started after about 30 seconds and persisted for few minutes after DETTOL was washed out.

In the fifth technique NEKO soap was studied. 7 volunteers were available for the test.

The viable count of the bacteria after 1/2 minute social wash varied from 700,000-33,600,000/100 mls. (12.75 - 47.06% of the original count). After further 4 1/2 minutes surgical scrub the count was 131,000- 450,000 (0.09 - 33.3% of the original count). No volunteer had any complaint about the use of the NEKO soap.

In this experiment due precautions were taken to counter-act the "CARRIED OVER" effect of mercuric iodide.

organisms and counting was not possible, hence result not reported.
The volunteer had burning on the dorsum of one hand after spirit rinsed.
In the fifth technique NEKO soap was studied. 7 volunteers were available for the test.
The residual count after social wash varied at 700,000-33,600,000/100 mls. (12.75 - 47.06% of the original count).
After surgical scrub for 4 1/2 minutes the count varied from 131,000- 450,000/100 mls. (0.09 - 33.3% of the original count).
Residual count after surgical scrub varied from 700,000-33,600,000/100 mls. (12.75 - 47.06% of the original count). Nobody had any reaction on the skin.
In the fourth technique DETTOL was studied.
3 volunteers were studied in this technique. 3 samples were collected like first and third samples of first technique.
Initial count was high in all volunteers (except in volunteer no. 10) probably due to summer and excessive perspiration and varied from 470,000-22,800,000/100 mls.
Percentage of residue after surgical scrub varied from 1.25-88.12% of the original count.
In the fourth technique which consisted rubbing a ml. of pure DETTOL in a standard way, after washing hands for 5 minutes with sunlight soap, for 30 seconds and allowing it to act for 5 minutes before washing.
The count varied from 4,500-1,375,000/100 mls. and the residual count varied from 0.08 to 10.58% of the original count.



DISCUSSION :-

Transmission of pathogenic organisms by fingers is an established fact, hence it is important to scrub pre-operatively.

Surgical scrub is not a complete safe-guard against infection as is seen from the result that after any washing technique a considerable number of bacteria persist on the hands. This shows that skin cannot be sterilised by these techniques and use of sterilised gloves is still necessary.

Although gloves are worn it is still important to have as clean hands as possible as glove puncture is very common.

Incidence of Glove Puncture

Devenish Miles	1939	24%
Penikett & Goril	1958	30%
PRESENT SERIES	1964	35%

The surgeon may not be aware of the small puncture, also, the glove is accidentally torn during operation then gross contamination of the wound may occur.

Even after 5 minutes scrub large number of organisms persist on the hands which stresses the importance of studying such problems and to find out some method which can be IDEAL.

Common organisms which could be transferred by hands are S. aureus, Coliform organisms, Ps. pyocyaneous.

Hands may be contaminated from various sources like septic dressings or from some part of the body, commonly anterior nares which harbour large number of bacteria. Many of the epidemics of post-operative sepsis by S. aureus have been traced to the nasal carriers.

Ronald Hare in 1958 stressed that sites other than nose like perineum, axilla, umbilicus and hands may be important sources for S. aureus; hence it is no longer justifiable to assume that only nasal carriers need be considered when attempts are made to trace the donor during the out break of infection.

In our series out of 12 volunteers only 3 were carrying coagulase positive staphylococci (25% carrier rate) in the nose.

Amongst the other sites investigated for carriage of S. aureus were back of the fore arms, cubital fossae and perineum.

One volunteer had S. aureus in the cubital fossae swab.

No volunteer was a perineal carrier of S. aureus.

These coagulase positive staphylococci were investigated for their anti-biotic sensitivity. Two of the strains were relatively resistant to the common antibiotics e.g. penicillin and streptomycin, sensitive to erythromycin and tetracycline while sensitivity to chloramphenicol was marked.

We kept records of sensitivity of organisms from cases of post-operative sepsis and the sensitivity pattern was similar.

Scrub brush should be sterile and kept in an anti-septic solution. It was found that the theatre nail brush was very heavily contaminated with coliform organisms.(See Photograph). Such brushes instead of cleaning increases the contamination of the hands.

DISCUSSION

Transmission of pathogenic organisms by hands is an established fact, hence it is important to scrub pre-operatively. Surgical scrub is not a complete anti-septic against infection as is seen from the results that allow any viable bacteria a considerable number of bacteria persist on the hands. This shows that skin cannot be sterilized by these techniques and use of sterilized gloves is still necessary. Although gloves are worn it is still important to have as clean hands as possible as gloves puncture is very common.

Incidence of Glove Lacerations

1950	1000	1000
1951	1000	1000
1952	1000	1000

The surgeon may not be aware of the small puncture, which the glove is accidentally torn during operation then cross contamination of the wound may occur. Even after 5 minutes scrub large number of organisms persist on the hands which stresses the importance of sterilizing gloves and to find out some method which can be used. Common organisms which could be transferred by hands are P. aureus, Coliform organisms, St. pyogenes.

Tap handles were found clean and did not harbour any pathogenic organisms, wash basins were also free from the pathogenic organisms. If tap handles are contaminated with pathogens than hands may be contaminated during beginning of hands washing or accidentally at the end, as they are operated by the surgeon himself.

If the basin is not clean than hands may be contaminated by splashing of the water from the basin during scrubbing.

Result of washing technique showed that hands were heavily contaminated and contamination varied from person to person. It also varied from the same person at different times. It was high during summer days probably due to excessive perspiration.

The reduction in the bacterial count after social wash was considerable in the majority of the volunteers as superficial organisms were very easily removed and washed out.

Occasional rise in the bacterial count after SOCIAL WASH, as in volunteer number 1 in technique 'A' and 'B', could be explained by the fact that the transient flora may be brought to the surface by the previous washing.

Contrary to the common belief, bacterial count after surgical scrub was not low to make operative procedure safe without gloves.

FALL IN THE BACTERIAL COUNT AFTER
SURGICAL SCRUB WITH SOAP.

Price	1938	Scrubbing time with soap & water - 6 minutes.	Reduction in Bacterial count..... 50%
Present series.	1964	Scrubbing time with soap & water - 5 minutes.	Reduction in Bacterial Count..... 75%

Tap handles were found clean and did not harbour any pathogenic organisms, wash basins were also free from pathogenic organisms. If tap handles are contaminated with pathogens than hands may be contaminated during beginning of washing or accidentally at the end, as they are operated by the surgeon himself.

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TABLE IN THE BACTERIAL COUNT AFTER SURGICAL SCRUB WITH SOAP.

Reduction in Bacterial Count.....	Scrubbing time with soap & water - 5 minutes.	1938	Price
Reduction in Bacterial Count.....	Scrubbing time with soap & water - 5 minutes.	Present series.	1964

Resident bacterial are characterised by persistence and variable diminution even after prolong washing and treatment with anti-septics. They include relatively few type of the bacteria and appear to colonise the skin; most of them are harmless, but amongst them may in some individual be found strains of *S. aureus* as pointed out by Evans, Smith, Johnston and Giblett(1930) Hare and Ridly (1958), Lowbury Lilly and Bull (1960).

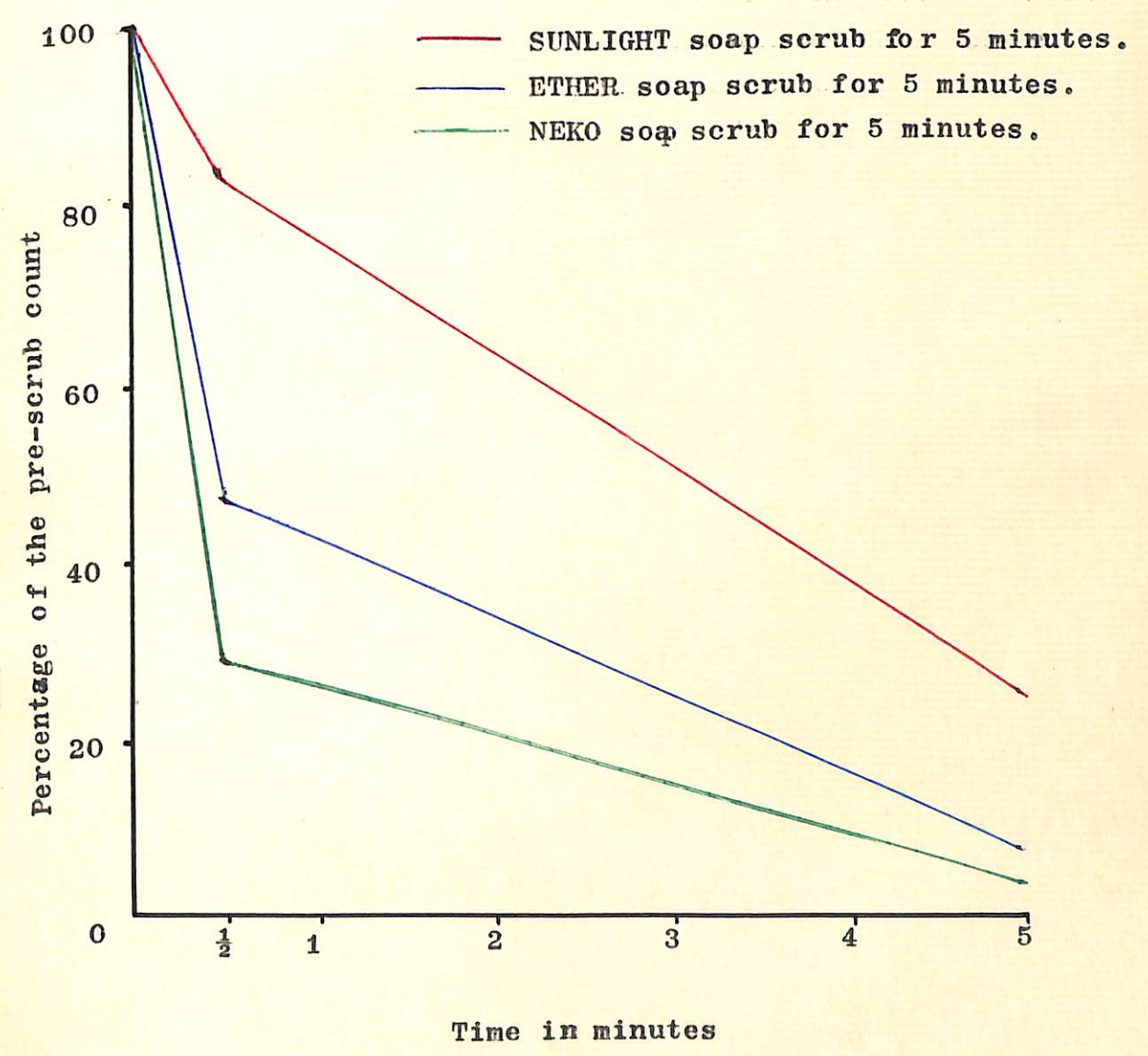
However the result of spirit rinse showed remarkable fall indicating that it is a good, quickly acting anti-septic to be used after surgical scrub. Absolute alcohol was used with the idea that water on the hands shall dilute it and the concentration shall be near about 70% w/w which has a maximum penetrating capacity. This has no real residual effect and the count within the gloves could be expected to rise during the operation.

Surgical scrub with ETHER soap showed the results superior to that of SUNLIGHT soap. ^{NEKO soap is better than Ether soap.} Some people are sensitive to ETHER soap but amongst the volunteers no-body had any reaction on the hands.

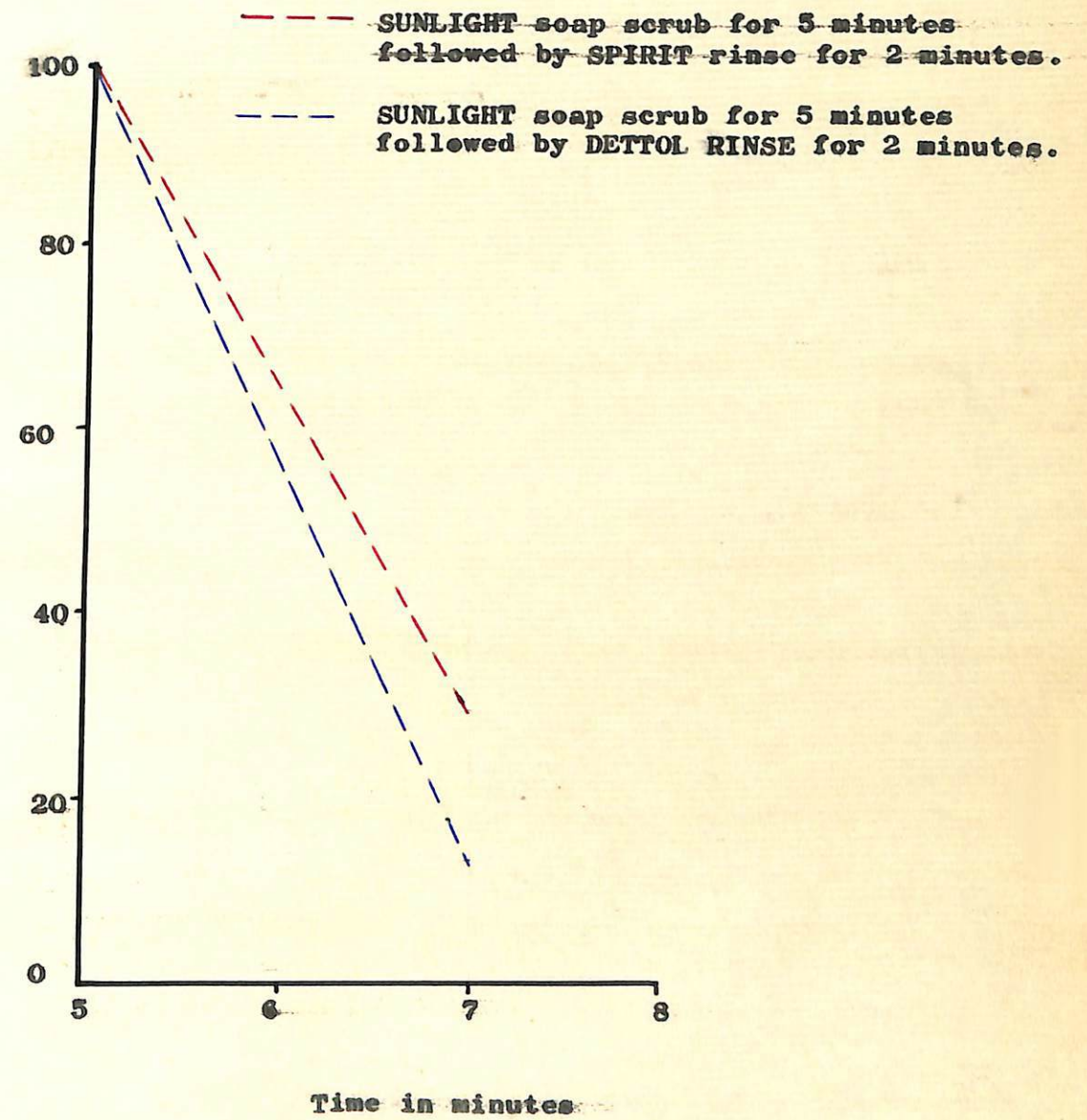
When the results of SPIRIT are compared with that of DETTOL, it seems that Dettol is better than Spirit (there may also be a residual effect to help).

Like Spirit and Dettol, I wanted to study the effect of the 'SAVLON' liquid anti-septic, but carried over effect of Savlon was remarkable, and to nullify its action by human plasma, egg and blood agar was futile.

In a personal communication with I.C.I. (India) Private Ltd., it was suggested to use 2% horse serum, 1% polysorbital 80; and 0.3% egg lecithin; but it was not possible to get them locally hence the plan was dropped.



Percentage of the viable bacterial count at the end of 5 minutes SUNLIGHT soap scrub.



CONCLUSIONS :-

1. Bacterial flora of the hands vary from person to person, and with the same person at different times. Some are heavy carriers while others carry small number of organisms to that much extent that the residual count after 5 minutes scrub is higher than the original count of the person having low count.

2. Of the 12 volunteers 3 volunteers (25%) were nasal carriers of *S. aureus* (coagulase test positive). From their sensitivity to anti-biotics it looks that they are hospital strains.

3. One of the volunteers showed one colony of *S. aureus* from the cubital fossae - probably a transient organism.

4. There was no volunteer who was a perineal carrier of *S. aureus*.

5. Fall in viable bacterial count at the end of SOCIAL WASH (30 seconds wash) was considerable; while fall at the end of surgical scrub was not markedly progressive.

6. Fall in viable bacterial count after SPIRIT rinse (preceded by 5 minutes scrub with sunlight soap) was worthy of note.

7. Ether soap is preferable to SUNLIGHT soap; NEKO soap superior to Ether soap.

8. Dettol was found superior to Spirit but more work should be done on a large scale to verify the result, with special precaution to exclude the CARRIED OVER effect of Dettol is necessary.

9. Nail brushes should be properly sterilised and kept in an anti-septic solution like 20% Dettol to avoid contamination.

10. Near each wash basin a sand operated hour glass (5 minutes) or a clock should be available.

11. Water supplied in winter for hand scrubbing should be warm otherwise washing time tends to be cut down.

*

CONCLUSIONS :-

1. Bacterial flora of the hands vary from person to person, and with the same person at different times. Some are heavy carriers while others carry small number of organisms to that much extent that the residual count after 5 minutes scrub is higher than the original count of the person having low count.
2. Of the 12 volunteers 3 volunteers (25%) were nasal carriers of S. aureus (coagulase test positive). From their sensitivity to anti-biotics it looks that they are hospital strains.
3. One of the volunteers showed one colony of S. aureus from the cubital fossae - probably a transient organism.
4. There was no volunteer who was a permanent carrier of S. aureus.
5. Fall in viable bacterial count at the end of SOCIAL WASH (30 seconds wash) was considerable; while fall at the end of surgical scrub was not markedly progressive.
6. Fall in viable bacterial count after SPIRIT WASH (preceded by 5 minutes scrub with sunlight soap) was worthy of note.
7. Ether soap is preferable to SUNLIGHT soap; WENO soap superior to Ether soap.
8. Betteol was found superior to Spirit but more work should be done on a large scale to verify the result, with special precaution to exclude the CARRIED OVER effect of Betteol is necessary.
9. Nail brushes should be properly sterilised and kept in an anti-septic solution like 20% Betteol to avoid contamination.
10. Wear each wash basin a sand operated hour glass (5 minutes) or a clock should be available.
11. Water supplied in winter for hand scrubbing should be warm otherwise washing time tends to be cut down.

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-: T A B L E :-

Stephylococcal carriage at various sites

Volun teer Number	Nasal Swab	Back of fore-arm		Cubital fossa.	Perineum
		Right.	Left		
1.	S.albus <i>+++</i> *S.aureus <i>++</i>	Misc. <i>+++</i> S.citreus <i>++</i>	Misc. <i>+++</i>	Misc. <i>++</i>	Misc. <i>+++</i> S.albus <i>+++</i>
2.	S.albus <i>+++</i> *S.aureus <i>+++</i>	S.citreus <i>++</i>	S.citreus <i>++</i>	S.citreus <i>++</i>	Misc. <i>++</i>
3.	S.albus <i>+++</i>	S.albus <i>++</i>	S.albus <i>++</i>	S.albus <i>+</i>	S.albus <i>+++</i>
4.	S.albus <i>+++</i>	S.albus <i>++</i>	S.albus <i>++</i>	S.albus <i>++</i>	S.albus <i>+++</i> Misc. <i>+++</i>
5.	S.albus <i>+++</i>	S.albus <i>+++</i>	S.albus <i>+++</i>	S.citreus <i>+++</i>	NOT GIVEN.
6.	S.albus <i>+++</i>	S.albus <i>++</i> S.citreus <i>++</i>	S.albus <i>+++</i> S.citreus <i>++</i>	S.citreus <i>++</i>	S.albus <i>+++</i> Misc. <i>+++</i>
7.	S.albus <i>+++</i>	S.albus <i>++</i>	S.citreus <i>+++</i>	S.albus <i>++</i>	Misc. <i>+++</i>
8.	S.albus <i>+++</i> *S.aureus <i>+++</i>	S.albus <i>++</i>	S.citreus <i>+++</i>	S.albus <i>++</i>	Misc. <i>+++</i> S.albus <i>+++</i>
9.	S.albus <i>+++</i>	S.albus <i>+++</i> S.citreus <i>+++</i>	S.albus <i>+++</i> S.citreus <i>+++</i>	S.albus <i>++</i> S.citreus <i>++</i>	S.albus <i>+++</i>
10.	S.albus <i>+++</i>	S.albus <i>+++</i> S.citreus <i>++</i>	S.albus <i>+++</i>	S.albus <i>+++</i>	S.albus <i>+++</i> S.citreus <i>++</i>
11.	S.albus <i>+++</i>	S.albus <i>+++</i>	S.albus <i>+</i>	S.albus <i>+</i>	S.albus <i>+++</i>
12.	S.albus <i>+++</i>	S.albus <i>++</i>	Misc. <i>++</i>	S.citreus <i>++</i> *S.aureus <i>+</i>	Misc. <i>+++</i> Misc. <i>+++</i>

* = Coagulase test positive
 + Number of colonies between 1 -10.
 ++ Number of colonies between 10-50.
 +++ Number of colonies more than 50.

Tap water 1 ml.inoculated - -no growth after 20 hours at 37°C
 Tank water 1 ml.inoculated no growth after 20 hours at 37°C
 Water from the jug - 1ml.inoculated after 20 hours at 37°C
profuse growth of anthracoid organism

O.T.1 Wash basin swab - 76 colonies predominant organisms-S.albus
 O.T.2 Wash basin swab - 27 colonies predominant organisms-S.albus
 O.T.1 Tap handle swab - 4 colonies predominant organisms-S.albus
 O.T.2 Tap handle swab - 20 colonies predominant organisms-S.albus
 O.T.1 Nail brush impression- Profuse growth of coli form organisms.

Results of Techniques I & II

Volunteer number	Sample - A	Sample - B	Sample - C	Sample - D
1	70,000	1,000,000 (142.9%)	5,000 (7.742%)	0 (0%)
2	590,000	460,000 (77.96%)	240,000 (40.67%)	118,000 (22.93%)
3	210,000	170,000 (82.83%)	112,000 (53.33%)	? Contaminated
4	-	-	-	-
5	920,000	740,000 (80.42%)	275,000 (29.88%)	112,000 (12.17%)
6	880,000	670,000 (76.14%)	640,000 (72.73%)	81,000 (9.204%)
7	3,500,000	650,000 (18.57%)	280,000 (8.000%)	35,000 (1.000%)
8	650,000	89,000 (13.70%)	62,000 (9.539%)	4,000 (0.6155%)
9	390,000	32,000 (8.204%)	24,000 (6.153%)	2,000 (0.5128%)
10.	1,220,000	1,220,000 (100.0%)	143,000 (11.71%)	37,000 (3.033%)
11	610,000	300,000 (48.06%)	240,000 (39.34%)	40,000 (6.558%)

- Sample - A. Sample before washing.
- Sample - B. 15 seconds soap scrub followed by 15 seconds wash in running tap water.
- Sample - C. Further 4½ minutes scrub with SUNLIGHT soap.
- Sample - D. Spirit rinse with 10 mls. of alcohol allowed to act for 2 minutes.

Figures indicates No. of viable bacteria /100 mls. of washing fluid.
 Figures in the brackets indicate percentages of the original count e.g. sample - A.

Technique - I :- SUNLIGHT soap scrub for 5 minutes.
 Technique - II :- Technique - I followed by 10 mls. of spirit rinse allowed to act for 2' minutes.

Results of Technique I & II

Volunteer number	Sample - A	Sample - B	Sample - C	Sample - D
1	70,000	1,000,000	5,000	0
2	590,000	480,000	240,000	118,000
3	310,000	170,000	143,000	118,000
4	-	-	-	Contaminated
5	930,000	740,000	278,000	113,000
6	880,000	670,000	640,000	81,000
7	3,200,000	650,000	280,000	32,000
8	680,000	88,000	82,000	4,000
9	390,000	32,000	24,000	2,000
10	1,320,000	1,320,000	143,000	37,000
11	610,000	300,000	240,000	40,000

Sample - A. Sample before washing.
 Sample - B. 15 seconds soap scrub followed by 15 seconds wash in running tap water.
 Sample - C. Further 4½ minutes scrub with SUNLIGHT soap.
 Sample - D. Spirit rinse with 10 mls. of alcohol allowed to set for 2 minutes.

Figures indicate number of viable bacteria per 100 mls. of washing fluid.
 Figures in the brackets indicate percentages of the original count e.g. sample - A.

Technique - I :- SUNLIGHT soap scrub for 5 minutes.
 Technique - II :- Technique - I followed by 10 mls. of spirit rinse allowed to set for 2 minutes.

Results of Technique - III

Volunteer number.	Sample - A	Sample - B	Sample - C
1	39,000	11,000 (28.20%)	1,000 (2.564%)
2	510,000	240,000 (47.06%)	140,000 (27.46%)
3	--	--	--
4	--	--	--
5	--	--	--
6	580,000	370,000 (63.89%)	270,000 (46.56%)
7	540,000	290,000 (53.70%)	Contaminated
8	--	--	--
9	540,000	129,000 (23.88%)	38,000 (7.037%)
10	102,000	68,000 (66.67%)	26,000 (25.49%)
11	1,810,000	840,000 (46.40%)	8,500 (0.469%)

Technique - III :- ETHER soap scrub for 5 minutes.

Sample A :- Sample before washing.
 Sample B :- Sample after 15 seconds Ether soap washing followed by 15 seconds washing in running tap water.
 Sample C :- Sample after further 4½ minutes scrub with Ether soap.

Figures indicate number of viable bacteria per 100 mls of washing fluid.
 Figures in the brackets indicate percentages of the original counts e.g. Sample - A.

Results of Technique - III

Volunteer number	Sample - A	Sample - B	Sample - C
1	39,000	11,000 (28.20%)	1,000 (2.56%)
2	310,000	240,000 (77.42%)	140,000 (45.16%)
3	---	---	---
4	---	---	---
5	---	---	---
6	280,000	370,000 (132.14%)	370,000 (132.14%)
7	240,000	390,000 (162.50%)	390,000 (162.50%)
8	---	---	---
9	240,000	122,000 (50.83%)	38,000 (15.83%)
10	102,000	88,000 (86.37%)	36,000 (35.39%)
11	1,810,000	840,000 (46.41%)	8,500 (0.47%)

Technique - III :- ETHER soap scrub for 5 minutes.

Sample A :- Sample before washing.

Sample B :- Sample after 15 seconds ether soap washing followed by 15 seconds washing in running tap water.

Sample C :- Sample after further 4 1/2 minutes scrub with ether soap.

Figures indicate number of viable bacteria per 100 ml of washing fluid.

Figures in the brackets indicate percentage of the original counts e.g. Sample - A.

Results of Technique - IV (D)

Volunteer number	Sample - A	Sample - B	Sample - C
1	645,000	295,000 (45.74%)	215,000 (33.3%)
2	23,700,000	7,110,000 (30.00%)	135,000 (1.78%)
3	---	---	---
4	3,740,000	1,760,000 (47.06%)	160,000 (4.28%)
5	---	---	---
6	---	---	---
7	3,640,000	880,000 (24.1%)	450,000 (12.70%)
8	135,000,000	23,600,000 (62.40%)	131,000 (23.81%)
9	---	---	---
10	1,330,000	830,000 (62.40%)	316,000 (23.81%)
11	5,500,000	700,000 (12.75%)	340,000 (6.20%)

Technique - IV :- NEKO Soap scrub for 5 minutes.

Sample 'A' :- Sample before washing.

Sample 'B' :- Sample after 15 seconds of NEKO soap washing followed by 15 seconds washing in running tap water.

Sample 'C' :- Sample after further 4 1/2 minutes scrub with NEKO soap.

Figures indicate number of viable bacteria per 100 ml of the washing fluid.

Figures in the brackets indicate percentage of the original count e.g. Sample - A.

Results of Technique - IV (D)

Volunteer number	Sample - A	Sample - B	Sample - C
1	645,000	225,000 (34.73%)	215,000 (33.33%)
2	22,700,000	7,110,000 (30.00%)	135,000 (1.78%)
3	--	--	--
4	2,140,000	1,780,000 (83.22%)	160,000 (4.28%)
5	--	--	--
6	--	--	--
7	2,610,000	880,000 (33.71%)	450,000 (17.24%)
8	132,000,000	23,600,000 (17.88%)	131,000 (0.31%)
9	--	--	--
10	1,220,000	830,000 (68.03%)	310,000 (25.41%)
11	2,500,000	700,000 (28.00%)	240,000 (9.60%)

Technique - IV :- NEKO soap scrub for 5 minutes.

Sample 'A' :- Sample before washing.

Sample 'B' :- Sample after 15 seconds of NEKO soap washing followed by 15 seconds washing in running tap water.

Sample 'C' :- Sample after further 4 1/2 minutes scrub with NEKO soap.

Figures indicate number of viable bacteria per 100 mls. of the washing fluid.

Figures in the brackets indicate percentage of the original count e.g. Sample - A.

Results of technique - V

Volunteer number.	Sample - A	Sample - B	Sample - C
1	1,030,000	390,000 (37.87%)	14,500 (1.406%)
2	1,510,000	560,000 (37.09%)	102,500 (6.787%)
3	3,030,000	2,550,000 (84.16%)	314,000 (10.35%)
4	2,880,000	2,480,000 (86.12%)	87,000 (3.091%)
5	25,600,000	9,100,000 (35.50%)	1,275,000 (4.976%)
6	7,150,000	2,250,000 (31.47%-app.)	725,000 (10.14%-app.)
7	3,880,000	1,850,000 (47.68%)	72,000 (1.856%)
8	--	--	--
9	--	--	--
10	470,000	23,000 (4.894%)	4,500 (0.957%)
11	1,690,000	182,000 (10.76%)	113,000 (6.686%)

Technique - V :- SUNLIGHT soap scrub for 5 minutes followed by DETTOL rinse for 2 minutes.

Sample 'A' :- Before washing.

Sample 'B' :- Sample after 15 seconds of NEKO soap washing followed by 15 seconds washing in running tap water.

Sample 'C' :- Sample after further 4 1/2 minutes scrub with NEKO soap.

Figures indicate number of viable bacteria per 100 mls. of the washing fluid.

Figures in the brackets indicate percentage of the original count e.g. Sample - A.



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