

Skin Irritation Caused by Alcohol-Based Hand Rubs

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1. Introduction

In the late 1990s and early part of the 21st century, alcohol-based hand rubs started to gain popularity. Today, alcohol-based hand rubs are widely used for infection control in clinical practice. However, many healthcare workers complain about unacceptable skin irritation caused by alcohol-based hand rubs. In spite of the complaint, when the irritant effect of alcohol on the skin has been evaluated, most authors found low toxicity (Boyce et al., 2000; de Haan et al., 1996; Lübbe et al., 2001; Winnefeld et al., 2000).

Kownatzki has pointed out that the skin irritation of healthcare workers is not simply caused by alcohol antiseptics but by combined damage resulting from the alcohol antiseptics dissolving lipids in the stratum corneum, the removal of lipids from the skin surface by detergent washing, and the skin becoming over-hydrated from wearing gloves.

To reduce the adverse effects of alcohol-based hand rubs, it is known that adding emollients or humectants is efficacious (Many studies are reviewed in Boyce & Pittet, 2002).

By contrast, addition of a certain type of chemical compound such as cationic antiseptics may cause irritation (Tsuji et al., 1993).

Thus, so-called “alcohol-based hand rubs” include wide variations of alcohol formulations. When we discuss the skin irritancy of alcohol-based hand rubs, we need to note the formulation of each testing sample and the type and concentrations of the alcohols, emollients, and antiseptic compounds contained.

To evaluate the skin irritancy of alcohol-based hand rubs in human, animal experiments such as Draize rabbit tests are quite useful. However, using experimental animals requires special techniques and facilities, and also have problem in animal protection.

Hence, alternatives to animal experiments have been developed in last decades. To predict the skin irritancy in human, *in vitro* skin irritation tests using three-dimensional human skin models are quite useful. The EU has accepted the *in vitro* skin irritation test using a human skin model as stand-alone test to determine the skin irritation potential of a substance (OECD TG 439). However, the *in vitro* skin irritation tests using human skin models cannot be used for high alcohol-content solutions, such as alcohol-based hand rubs. To overcome this problem, the author has developed a novel *in vitro* evaluation method named “Skin model blowing method (SMBM)” (Yamamoto et al., 2010).

The first objective of this review is to summarize the structure and barrier function of the skin, the mechanism and evaluation methods of skin irritation, and the irritancy of alcohol-based hand rubs. The second objective is to implement the novel *in vitro* evaluation method “SMBM” for assessing the skin irritation caused by alcohol-based hand rubs, and show the evaluation results of some of the alcohol-based hand rubs used in Japan.

2. Structure and barrier function of the skin

2.1 Structure of the skin

The skin is the largest human organ and consists of two main layers: epidermis and dermis (Fig.1). One major task of the skin is to protect the organism from water loss and mechanical, chemical, microbial, and physical influences. The protective properties are provided by the outermost layer of the skin, the epidermis. The epidermis is approximately 100 to 150 micrometers thick, has no blood flow and includes the superficial layer known as the stratum corneum (Fig.2).

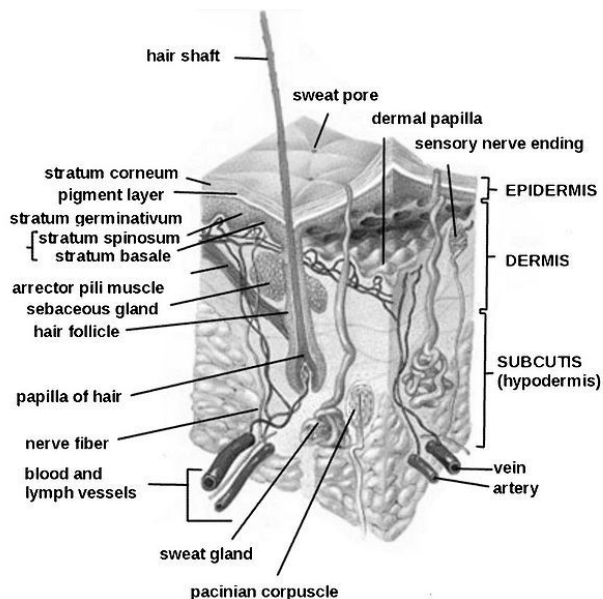


Fig. 1. Histological structure of the human skin¹

The stratum corneum consists of slabs of flat, platelike dead cells called corneocytes. The corneocytes, which are anucleated cells derived from keratinocytes, have no viable function and are called "dead" cells. They are continuously being sloughed off and then replaced in cycles of 3 to 4 weeks. The cells are pushed up from the living layer just lying below. The corneocytes are embedded in the intercellular lipid matrix, thus the structure of the stratum corneum can be roughly described by a "brick and mortar" model (Elias, 1983).

¹ U.S. National Cancer Institute, In: *Anatomy of the Skin*, Available from <http://training.seer.cancer.gov/melanoma/anatomy/>

2.2 Barrier function of the skin

The major factor that keeps the skin moist and pliable is the presence of intercellular lipids. These form a lamellar (stacked bilayers) structure surrounding the corneocytes and incorporate water into the stratum corneum. The lipids are derived from lamellar granules, which are released into extracellular spaces from degrading cells in the granular cell layer; and the membranes of these cells also release lipids, including cholesterol, free fatty acids and sphingolipids. Ceramide, a type of sphingolipid, is mainly responsible for generating the stacked lipid structures that trap water molecules in their hydrophilic region.

These lamellar lipids surround the corneocytes and form a semi-permeable barrier that prevents water and natural moisturizing factors (NMF) from moving out from the surface layers of the skin.

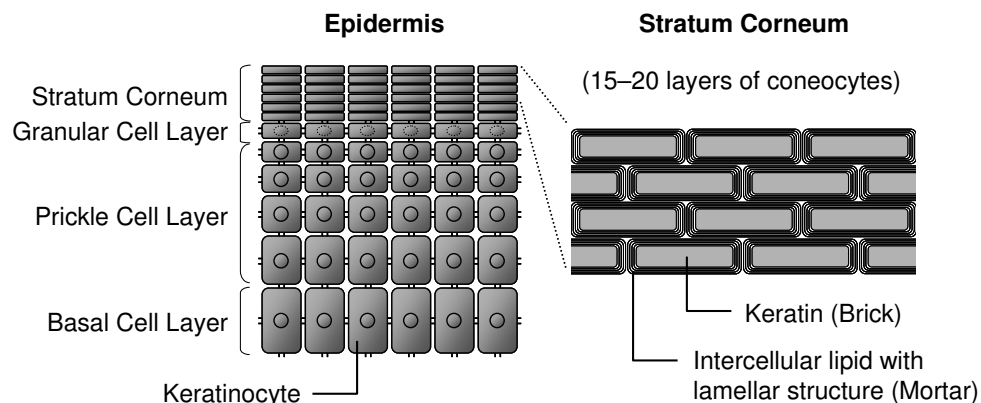


Fig. 2. Schematic diagrams of the epidermis and stratum corneum based on the “brick and mortar” model (Elias, 1983)

2.3 Measurement of the skin barrier function

2.3.1 Transepidermal water loss (TEWL)

The measurement of transepidermal water loss (TEWL) is an important non-invasive method for assessing the barrier function of the stratum corneum. As a consequence, TEWL has been found to be a very useful index for studying skin irritation induced by various physical and chemical effects. Exposure of the skin to chemicals (detergents) and physical conditions (occlusion and stripping) generally results in an increase of TEWL (Barel & Clarys, 1995).

Several TEWL measuring instruments such as Evaporimeter EP-2 (ServoMed, Sweden), Tewameter TM 300 (Courage+Khazaka electronic GmbH, Germany) and VapoMeter (Delfin Technologies, Finland) are commercially available. Evaporimeter and Tewameter are based on the open chamber system with two humidity and temperature sensors for measuring the water evaporation gradient at the surface of the skin.

By contrast, VapoMeter is based on the closed chamber system, and is easier to use than the open chamber device. However, its tendency to become saturated under high water

loss conditions could be a disadvantage when assessing dynamic TEWL (Cohen et al., 2009). Tewameter is able to detect significantly smaller differences than VapoMeter (de Paepe et al., 2005).

2.3.2 Electrical characteristics of skin surface

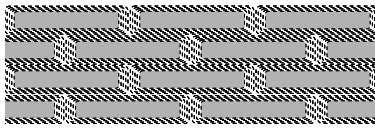
Deterioration of the skin barrier function leads to reduced hydration levels of the skin surface. To determine the hydration level of the skin surface, Corneometer CM 825 (Courage+Khazaka electronic GmbH, Germany) and SKICON-200EX (I.B.S Co., Ltd., Japan) are widely used. Corneometer CM 825 measures the changes in the dielectric constant caused by skin surface hydration by measuring changes in capacitance with a precision capacitor. SKICON-200EX measures high frequency conductance of the skin, which is sensitively correlated to the skin surface water content.

3. Mechanism of skin irritation

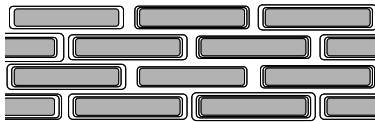
3.1 Inflammatory response

Foreign materials (e.g., micro-organisms, surfactants, etc.) that have penetrated the stratum corneum barrier encounter living epidermal cells. Interactions with keratinocyte surface molecules or membrane lipids activate the cells. Cytokines are released, emitting signals requesting assistance to blood vessels and white blood cells. Activation of Langerhans cells initiates an immune response, which is particularly effective when a given foreign material is encountered repeatedly. When these responses exceed a certain level, inflammatory symptoms are elicited (Gallin et al., 1992).

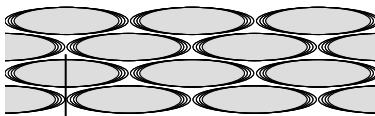
1. Destruction of the lipid lamellar structure



2. Loss of intercellular lipids



3. Over-hydration



Intercellular pools of water

Lower barrier function
(enhanced permeability)
of stratum corneum

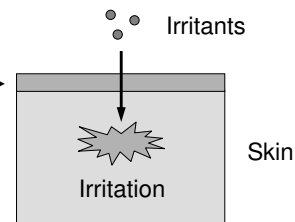


Fig. 3. Diagrams of structurally altered stratum corneum. Due to the deterioration of the barrier function (enhanced permeability) of epidermis, irritants can penetrate through the stratum corneum.

3.2 Hand hygiene and skin barrier function

The mechanism of skin barrier damage in healthcare workers was summarized by Kownatzki (Kownatzki, 2003). The main concern in hygiene-dependent risks to the skin's health is damage to the lipid barrier. The lipid barrier is jeopardized on three occasions: when the lipid lamellar structure is destroyed, the intercellular lipid is lost, and the skin is over-hydrated. In healthcare settings, these phenomena usually occur in a concerted situation of alcohol antiseptics, detergent cleaning, and glove work.

Destruction of the lipid lamellar structure

Antiseptic alcohols, which are organic solvents, are capable of dissolving stratum corneum lipids and destroying the barrier. Alcohol remaining on the skin evaporates leaving the lipids on the skin, but the lipids do not reassume the original structure and arrangement of the barrier and do lose the sealing function.

Loss of intercellular lipids

Detergents clean surfaces by removing lipids, together with any adhering contaminants. Sebum lipids on the skin surface, which are encountered and emulsified first by detergents, may provide protection for the underlying barrier lipids. Repeated detergent washes and progressive removal of surface lipids reduce the lipid-dependent cleaning efficiency and allow the detergent molecules to penetrate deep in the stratum corneum. In individuals with less supply of sebum lipids, this occurs more quickly.

Over-hydration

There is a high rate of hand problems among professions whose hands have frequent contact with water or wet objects such as food workers and hair dressers. Also the gloves worn by healthcare workers create a wet environment as they do not allow the sweat to evaporate. Extended water exposure leads to extensive disruption of stratum corneum intercellular lipid lamellae. The hydration induces disruption of the intercellular lipid lamellae, forms large pools of water in the intercellular space and creates corneocyte separations (Warner et al., 2003).

4. *In vivo* and *in vitro* evaluation of skin irritation

Human patch testing is commonly used to evaluate the skin irritation caused by a substance. Animal and *in vitro* testing is also utilized to predict the skin irritancy in human.

4.1 Human testing (single-application patch test)

Widely used method for assessing skin irritation include single-application patch testing, cumulative irritation test, chamber scarification test and immersion tests (Levin & Maibach, 2004). Especially, many variations of single-application patch test have been developed. Testing is often performed on undiseased skin (Skog, 1960) of the dorsal upper arm or back. The required test area is small, and up to ten materials can be tested simultaneously and compared. A reference irritant substance is often included to interpret variability in test responses. In general, screening of new materials involves open application on the back or dorsal upper arm for a short time (30 min to 1 hr) to minimize potential adverse events in the subjects.

The National Academy of Sciences (National Academy of Sciences and Committee for the Revision of NAS Publication 1138, 1977) recommended a 4-hr single-application patch test protocol for routine testing of skin irritation in humans. In general, patches are occluded onto the dorsal upper arm or back skin of patients. The degree of occlusion varies according to the type of occlusive device; the Hilltop or Duhring chambers or an occlusive tape will enhance percutaneous penetration as compared to a non-occlusive tape or cotton bandage (Patil et al., 1996). Potentially volatile materials should always be tested with a non-occlusive tape.

Exposure time to the putative irritant varies greatly, and is often customized by the investigator. Volatile chemicals are generally applied for 30 min to 1 hr while some chemicals have been applied for more than 24 hr.

Following patch removal, the skin is rinsed with water to remove the residue. Skin responses are evaluated 30 min to 1 hr following patch removal in order to allow hydration and pressure effects of the patch to subside. Another evaluation is performed 24 hr following the patch removal. The animal Draize scale is used to analyze test results (see Table 1). The Draize scale does not include papular, vesicular, or bullous responses; and other scales have been developed to address these needs.

Single-application patch tests generally heal within one week. Depigmentation at the test site results in some subjects.

Erythema	
No erythema	0
Slight erythema	1
Well-defined erythema	2
Moderate or severe erythema	3
Severe erythema or slight eschar formation (injuries in depth)	4
Edema	
No edema	0
Very slight edema	1
Slight edema (well-defined edges)	2
Moderate edema (raised >1 mm)	3
Severe edema (raised >1 mm and extending beyond the area of	4

Table 1. Draize scoring system

4.2 Animal testing (Draize rabbit test)

In order to evaluate the skin irritation, Draize rabbit test, guinea pig immersion test and mouse ear test are utilized as animal models. Especially, the Draize scores are most accurate when compared to related compounds with a record of human exposure (Levin & Maibach, 2004).

The Draize rabbit test was developed in 1944, and has since been adopted in the US Federal Hazardous Substance Act (Patrick & Maibach, 1989). The test involves two (1 square inch) test sites on the dorsal skin of six albino rabbits. One site is abraded (through use of a hypodermic needle across the rabbit skin) and the other site remains intact. The stratum corneum is broken on the abraded site, without loss of blood. The undiluted "irritant" materials (0.5 g for solids or 0.5 ml for liquids) are placed on a patch and applied to the test sites. They are secured with two layers of surgical gauze (1 square inch) and tape. The animal is wrapped in cloth so that the patches are secure for a 24-hr period. Assessment of erythema and edema, utilizing the scale noted in Table 1, takes place 24 hr and 72 hr following patch application. Severe reactions are again assessed on days 7 or 14. Radiolabeled tracers or biochemical techniques to monitor skin healing is also utilized by some investigators. Other investigators supplement with histological evaluation of skin tissue (Mezei et al., 1966; Murphy et al., 1979).

The Draize test ultimately quantifies irritation with the primary irritation index (PII), which averages the erythema and edema scores of each test site and then adds the averages together. Materials producing a PII of <2 are considered nonirritating, 2-5 mildly irritating, and >5 severely irritating and require precautionary labelling. Subsequent studies have demonstrated that the PII is somewhat subjective because the scoring of erythema and edema require clinical judgment (Patil et al., 1998).

Main critics of the Draize test oppose the harsh treatment of animals. They argue that the Draize test is unreliable at distinguishing between mild and moderate irritants. Furthermore, they believe the Draize is not an accurate predictor of skin irritancy as it does not include vesiculation, severe eschar formation or ulceration in evaluating the PII. Finally, they argue that the Draize procedure is not reproducible (Weil & Scala, 1971) and they question its relevance with regard to human experience (Edwards, 1972; Nixon et al., 1975; Shillaker et al., 1989). Proponents of the Draize test point out that the test is somewhat inaccurate but it generally overpredicts the severity of skin damage produced by chemicals, and thereby errs on the side of safety for the consumer (Patil et al., 1996). This topic is still being hotly debated. For the meantime, the Draize assays are recommended by regulatory bodies.

4.3 *In vitro* testing (human skin models)

4.3.1 Overview of human skin models

Animal experiments such as Draize rabbit tests are quite useful for determining the skin irritancy in human. However, using experimental animals requires special techniques and facilities, and also have problem in animal protection. Three-dimensional human skin models and cultured human skin models, which have been proposed for therapeutic purpose of a full thickness skin defect resulting from burn or trauma, can be used to replace animal-based irritative studies. The human skin models have been developed during the last decades (Green et al., 1979; Bell et al., 1981; Asselineau et al., 1985). The first skin model was proposed by Green et al. in 1979, who made an artificial epidermis from human epidermal keratinocytes. This type of human skin model is called "reconstructed human epidermis (RhE)". The skin model consisting of dermis and epidermis which resembled the real human skin was reported by Bell et al. in 1981. Various human skin models have been developed thereafter and are commercially available today (Table 2).

Product Name	Structure	Manufacturer
EpiDerm	Epidermis	MatTek Corp.
EpiDermFT	Epidermis on dermis	MatTek Corp.
EPISKIN	Epidermis on collagen gel	SkinEthic Laboratories
SkinEthic RHE	Epidermis	SkinEthic Laboratories
EST-1000	Epidermis	CellSystems Biotechnologie Vertrieb GmbH
TESTSKIN	Epidermis on dermis with collagen gel	TOYOBO Co., Ltd.
Vitrolife-Skin	Epidermis on dermis with collagen sponge	GUNZE Ltd.
LabCyte EPI-MODEL	Epidermis	Japan Tissue Engineering Co., Ltd.

Table 2. Commercially available human skin models

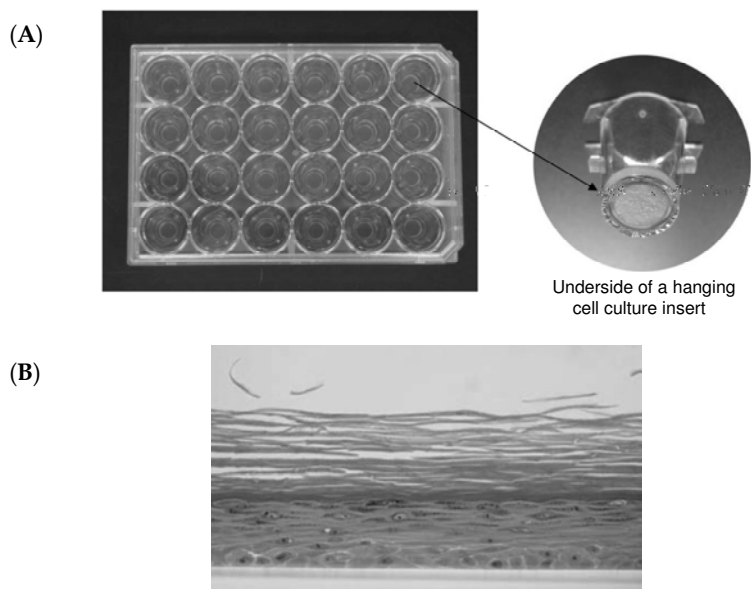


Fig. 4. An example of human skin model (LabCyte EPI-MODEL 24).² (A) Appearance of the skin model in hanging cell culture insert in 24-well microplate. (B) Histological cross-sectional view of the skin model with H&E staining. Epidermal cells were located on a microporous membrane.

² Photographs by courtesy of Japan Tissue Engineering Co., Ltd.

4.3.2 *In vitro* evaluation of skin irritation by using human skin models

To evaluate and predict the skin irritancy in human, *in vitro* skin irritation tests using human skin models have been developed. During the development processes, appropriate endpoints for skin irritancy evaluation have been determined. Triglia et al. compared four endpoints on their dermal model: 1) cell viability determination with neutral red (NR), 2) cell viability determination with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT), 3) release of prostaglandine E₂ (PGE₂), and 4) release of lactate dehydrogenase (LDH) (Triglia et al., 1991). They tested 13 chemicals, but there were no significant differences among the results of the four endpoints. Morota et al. compared six endpoints: 1) cell viability with MTT, 2) cell viability with NR, 3) release of PGE₂, 4) LDH, 5) interleukin-1 α (IL-1 α), and 6) interleukin-8 (IL-8) (Morota et al., 1999). They concluded that cell viability assays revealed good correlations with animal testing (Draize score of skin irritancy) and were advantageous to the other endpoints as they were easier to use and less costly. Recently, the EU has accepted the *in vitro* skin irritation test using RhE as stand-alone test to determine the skin irritation potential of a substance (OECD TG 439). In this guideline, cell viability assay with MTT is adopted.

However, *in vitro* skin irritation tests using human skin models have some limitations. For example, they cannot be used for samples containing high concentrations of ethanol. It is because most skin models are more sensitive to alcohols than the skin *in vivo*. Instead of the low irritation scores demonstrated in Draize rabbit skin tests, ethanol showed high toxicity in human skin model tests. In a dose-response test, higher concentration of ethanol resulted in lower cell viability (Genno et al., 1998; Li et al., 1991). It was found that ethanol concentrations above 30% affected the skin model, but had minimal effects on the rabbit skin.

Cytotoxicity of ethanol in human skin models are affected not only by the concentration of ethanol but also by the time of exposure. From the time course change of cytotoxicity test, it was shown that cell viability was not affected by short time exposure to ethanol (Nagasawa et al., 2002). Cell viability was found to be negligible when the skin was exposed to 76.9–81.4 vol% of ethanol for a period shorter than 1 minute (Yamamoto et al., 2010).

5. Irritancy and antimicrobial activity of alcohol-based hand rubs

Most alcohol-based hand rubs contain either ethanol, isopropanol or n-propanol, or a combination of two of them. Assessments of alcohol effects on the skin have involved evaluating the effects of individual alcohol at various concentrations, combinations of two or more alcohols, and alcohol solutions containing thickening agents, foaming agents, and/or small amounts of antiseptics.

5.1 Irritancy of alcohol

Most irritancy assessments of alcohol have shown that alcohols are little toxic to the skin (Boyce et al., 2000; de Haan et al., 1996; Lübbe et al., 2001; Winnefeld et al., 2000). However, many healthcare workers complain about unacceptable skin irritation caused by alcohol-based hand rubs. Even in the Guideline for Hand Hygiene in Healthcare Settings of the Centers for Disease Control (Boyce et al., 2002), skin tolerability of alcohol-based hand rubs is stated as potentially problematic: 'Although alcohols are among the safest antiseptics available, they can cause dryness and irritation'.

According to the well-designed patch testing with alcohols and sodium lauryl sulphate (SLS) as a model detergent, it was found that alcohols lead to only minor skin barrier changes and cause no changes in erythema independent of the concentration tested (Löffler et al., 2007). Compared to alcohols, the detergent SLS induced a much stronger barrier disruption and a pronounced skin hydration decrease.

Kownatzki has pointed out that the skin irritation of healthcare workers is not simply caused by alcohol antiseptics but by combined damage resulting from the alcohol antiseptics dissolving stratum corneum lipids, the removal of lipids from the skin surface by detergent washing, and the skin becoming over-hydrated from wearing gloves.

5.2 Basic formulation of alcohol-based hand rubs

Antimicrobial activity of alcohols results from their ability to denature proteins. Alcohol solutions containing 60–80% alcohol are most effective, with higher concentrations being less potent (Price, 1938; Harrington & Walker, 1903). This paradox results from the fact that proteins are not denatured easily in the absence of water (Larson & Morton, 1991). The alcohol content of solutions may be expressed as a percentage by weight, which is not affected by temperature or other variables, or as a percentage by volume, which may be affected by temperature, specific gravity and reaction concentration. For example, 70% alcohol by weight is equivalent to 76.8% by volume if prepared at 15°C, and 80.5% if prepared at 25°C (Price, 1938). In the Japanese Pharmacopoeia, ethanol solution for disinfection is defined as the concentration of 76.9–81.4% by volume.

5.3 Gel and foam formulations

Alcohol-based hand rubs intended for use in hospitals are available as low viscosity rinses, gels, and foams. For example, thickening agents such as polyacrylic acid or cellulose derivatives are commonly formulated in alcohol gels to increase the viscosity of alcohol solutions.

Limited data are available regarding the relative efficacy of various formulations. One field trial demonstrated that an ethanol gel was slightly more effective than a comparable ethanol solution in reducing bacterial counts on the hands of healthcare workers (Ojajärvi, 1991). However, a more recent study indicated that rinses reduced more bacterial counts on the hands than the gels tested (Kramer et al., 2002). Further studies are warranted to determine the relative efficacy of alcohol-based rinses and gels in reducing transmission of healthcare-associated pathogens.

In prospective trials, alcohol-based gels containing humectants caused significantly less skin irritation and dryness than the soaps or antimicrobial detergents tested (Boyce et al., 2000; Newman & Seitz, 1990).

5.4 Antiseptics formulation

Some alcohol-based hand rubs contain antiseptics in order to provide persistent (residual) activity. Addition of antiseptics (e.g., chlorhexidine or quaternary ammonium compounds) to alcohol-based formulations can result in persistent activity (Rotter, 1999).

Chlorhexidine, a cationic bisbiguanide, was developed in the United Kingdom in the early 1950s. It is effective against grampositive bacteria and has substantial residual activity.

Chlorhexidine base is barely soluble in water, and thus the water-soluble digluconate form (CHG) is widely used. Addition of low concentrations (0.5–1%) of chlorhexidine to alcohol-based preparations results in significantly greater residual activity than alcohol alone (Aly & Maibach, 1979; Lowbury et al., 1974).

Quaternary ammonium compounds are composed of a nitrogen atom linked directly to four alkyl groups, which may vary considerably in their structure and complexity (Merianos, 1991). Among this large group of compounds, alkyl benzalkonium chlorides (BAC) are the most widely used as antiseptics.

In Japan, alcohol-based hand rubs containing CHG or BAC are widely used in healthcare settings. For example, WELPAS (0.2% w/v BAC, 70% ethanol solution) and WELLUP (0.2% w/v CHG, 70% ethanol solution) are recommended for hand hygiene in the Guideline for the prevention of healthcare-associated infection in urological practice in Japan (Hamasuna et al., 2011).

Compared with CHG, BAC shows stronger activity to various microorganisms (Jono et al., 1985; Shimizu et al., 2002). However, alcohol-based hand rubs containing CHG are less irritative to the skin than those containing BAC (Tsuji et al., 1993). The skin irritancy level of alcohol-based hand rubs containing antiseptics correlates with the irritancy of the antiseptic compound contained (Tsuji et al., 1996).

It is known that alcohols may enhance skin permeation. For example, the enhancement ability of ethanol is maximized at the concentration of 50–70% (Kim et al., 1996; Watkinson et al., 2009). Hence the irritancy of antiseptic compounds may be amplified in alcohol-based hand rubs.

6. Reduction of skin irritancy

In prospective trials, alcohol-based hand rubs containing humectants caused significantly less skin irritation and dryness than the soaps or antimicrobial detergents tested. These results suggest that addition of humectants can minimize the skin irritation and dryness.

6.1 Humectants

Most alcohol-based hand rub formulations contain humectants (or emollients). The drying effect of alcohol can be reduced or eliminated by adding 1%–3% glycerol or other skin-conditioning agents (Many studies are reviewed in Boyce & Pittet, 2002). Moreover, in several recent prospective trials, alcohol-based rinses or gels containing emollients caused substantially less skin irritation and dryness than the soaps or antimicrobial detergents tested (Winnefeld et al., 2000; Boyce et al., 2000; Larson et al., 2001a, 2001b).

These studies, which were conducted in clinical settings, used various subjective and objective methods for assessing skin irritation and dryness. Further studies are warranted to know whether products with different formulations would yield similar results or not.

6.2 Skin barrier stabilizers

Lamellar structures of intercellular lipid in the stratum corneum are quite important to maintain the barrier property of the skin. It is known that some kind of compounds can stabilize the lamellar structures.

Ceramide

Ceramides are characteristic components of intercellular lipids in the stratum corneum. The lamellar structures of intercellular lipids are stabilized by long-chain ceramides. Alcohol-based hand rubs containing synthetic pseudo-ceramide are less likely to roughen the skin of the hands in comparison with hand rubs containing no emollient (Tsuboi et al., 2006).

MPC polymers (Lipidure®)

MPC polymers are novel phospholipid-like synthetic polymers composed of 2-methacryloyloxyethyl phosphorylcholine (MPC). They are biomimetic materials which have excellent biocompatibility as its structure closely resembles that of cell membrane phospholipids (Iwasaki & Ishihara, 2005). Recently, unique functions of the MPC polymers have been reported.

MPC homo-polymer can protect the barrier property of the stratum corneum by preventing the intercellular lipid bilayer (ILB) structure from being disrupted by extensive skin hydration (Lee, 2004). It helps maintain the barrier property of the skin by preventing disruption of the ILB structure, and functions as a barrier-like membrane to prevent toxic substances from penetrating into the skin.

The effects of MPC/n-butyl methacrylate (BMA) co-polymer on the water barrier function and water-holding capacity of the stratum corneum were examined by measuring transepidermal water loss (TEWL) and electrical conductance of the skin surface (Kanekura et al., 2002). The MPC/BMA co-polymer reduced TEWL in laboratory mice significantly compared with the control. Human skin treated with this polymer showed significantly greater ability to retain water at all time points.

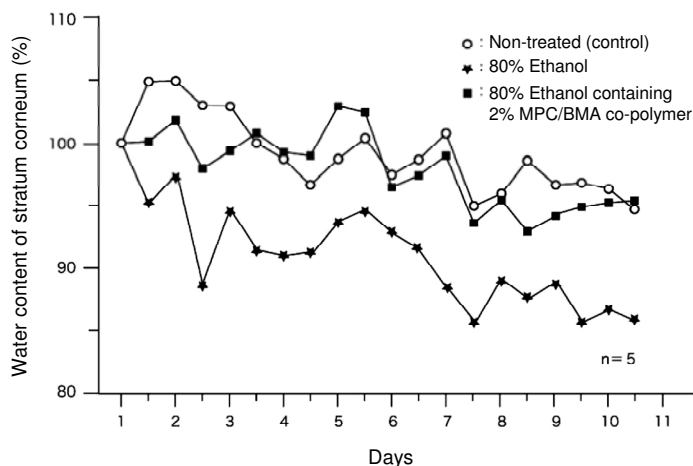


Fig. 5. Water content in the stratum corneum of hairless mice. A 100 μ L solution (80% ethanol or 80% ethanol + 2% MPC/BMA co-polymer) was applied on the back skin of hairless mice twice a day for 10 days. (Figure modified from Andoh et al., 2008)

The skincare function of the MPC/BMA co-polymer was also determined by Andoh. As shown in Fig. 5, both the control and the ethanol solution containing MPC/BMA co-

polymer showed the same tendency. By contrast, applying ethanol solution without MPC/BMA co-polymer decreased the water content of the stratum corneum. In addition, the presence or absence of MPC/BMA co-polymer had no relation to the bactericidal activity of the ethanol solutions (Andoh et al., 2008).

Recently, the unique function of MPC/stearyl methacrylate (SMA) co-polymer has been reported. It was found that the MPC/SMA co-polymer forms a self-assembled mosaic lamellar structure, which is structurally similar to ILB, by simple drying process. It is considered that the MPC/SMA co-polymer has a potential to act as an artificial intercellular lipid for damaged skin (Yamamoto et al., 2007).

Commercially available MPC polymers for skincare products are shown in Table 3.

Chemical Structure	Product Name
MPC* homo-polymer	Lipidure®-HM
MPC/BMA** co-polymer	Lipidure-PMB®
MPC/SMA*** co-polymer	Lipidure®-S
Polyol solution of MPC/SMA co-polymer	Lipidure®-NR

Note: *MPC: 2-methacryloyloxyethyl phosphorylcholine. **BMA: n-Butyl methacrylate. ***SMA: Stearyl methacrylate.

Table 3. Commercially available MPC polymers for skincare products³

7. *In vitro* evaluation of skin irritation caused by alcohol-based hand rubs

Animal experiments are quite useful for estimating the skin irritation potential in human. However, using experimental animals requires special techniques and facilities, and also has problem in animal protection. Thus development of an alternative to animal experiments is important not only from the viewpoint of ethical aspects but also for efficient research and development. The *in vitro* reconstructed human epidermis (RhE) has been applied for evaluating the skin irritancy of various substances. However, RhE has not been used for the evaluation of alcohol-based hand rubs because of the high skin permeability and cytotoxicity of alcohols. Recently, the author has developed a novel *in vitro* experimental method named "Skin model blowing method" (SMBM), which mimics the actual usage of alcohol-based hand rubs: putting on, spreading, rubbing into the skin, and drying. The skin irritation potential of alcohol-based hand rubs could be estimated by using SMBM. In this section, details of SMBM and evaluation results of some of alcohol-based hand rubs used in Japan are described.

7.1 Development of *in vitro* evaluation method using RhE

7.1.1 Experimental

Alcohol-based hand rubs used in this study

The alcohol-based hand rubs used in this study are summarized in Table 4.

³ <http://www.nof.co.jp/business/life/lipidure/english/>

Lipidure and Lipidure-PMB are registered trademarks of NOF Corporation in the U.S., and are registered trademarks or trademarks in other countries. All other product names are registered trademarks or trademarks of each company.

Code	Product Name	Antiseptics**	Supplier***
a	Ethanol for disinfection*	-	Kozakai
b	ISODINE PALM	PVP-I 0.5%	Meiji
c	WELLUP	CHG 0.2%	Maruishi
d	WELPAS	BAC 0.2%	Maruishi

Note: *Complying with the Japanese Pharmacopoeia (76.9–81.4 vol% of ethanol). **Antiseptics: Povidone iodine (PVP-I), Chlorhexidine gluconate (CHG), Benzalkonium chloride (BAC). ***Supplier: Kozakai Pharmaceutical Co., Ltd., Meiji Seika Pharma, Ltd., Maruishi Pharmaceutical Co., Ltd.

Table 4. Alcohol-based hand rubs (76.9–81.4 vol% of ethanol) used for studies

Reconstructed human epidermis (RhE)

The RhE kit LabCyte EPI-MODEL was purchased from Japan Tissue Engineering Co., Ltd.

Blowing equipment

Blowing equipment consisting of an air pump (exhaust volume: 1.3 L/min), tube and 4-channel nozzle (VACUBOY adapter, Integra Bioscience AG) was assembled in house.

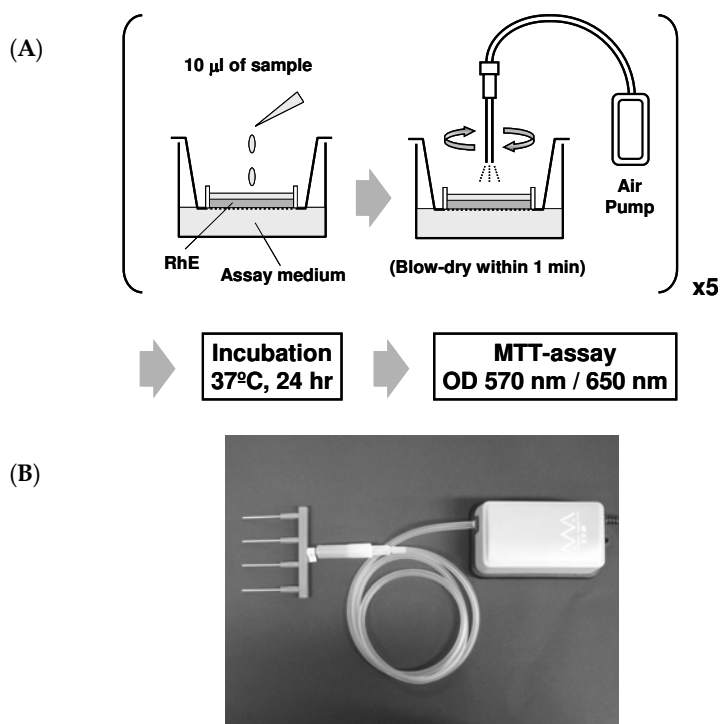


Fig. 6. Testing protocol and the blowing equipment. (A) Schematic illustration of testing protocol named "Skin model blowing method". (B) Blowing equipment consisting of an air pump (exhaust volume: 1.3 L/min), tube and 4-channel nozzle. The 4-channel nozzle corresponds to the tandem 4 epidermis models in 24-well microplate.

In vitro evaluation of skin irritancy, "Skin model blowing method" (SMBM)

Ten μL each of alcohol-based hand rub was applied to the surface of RhE, and blow-dried within 1 minute by using blowing equipment. This operation was repeated 5 times. As a control, only blow-drying was applied. After the operation, the RhE was incubated in an assay medium for 24 hr at 37°C in 5% CO_2 atmosphere. Then the RhE were further incubated in a MTT medium (0.5 mg of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide in assay medium) for 3 hr at 37°C in 5% CO_2 atmosphere. Living cells were dyed with purple formazan. The dyed RhE was put into microtube; then 200 μL of isopropyl alcohol was added to extract purple formazan. The extracts were measured for the absorbance at 570 nm (reference wavelength 650 nm) using a microplate reader (SpectraMax 250, Molecular Devices). Three RhE were used per group ($n=3$).

Comparative analysis between *in vivo* and *in vitro* experiments

The cell viability values obtained from SMBM and the integrated scores of irritation index of skin in rabbit (Tsuji et al., 1993) was compared. The integrate scores of **primary** irritation index were a: 1.2, b: 1.7, c: 5.7, and d: 58.3. The integrate scores of **cumulative** irritation index were a: 7.0, b: 6.5, c: 22.5, and d: 104.0.

Statistical analysis

Values were represented in means \pm SD. Experimental groups were compared with the control using Student's t-test. $P < 0.05$ and $P < 0.01$ were taken to be the level of statistical significance.

7.1.2 Results

In vitro evaluation of skin irritancy by SMBM

The cell viability of the RhE exposed to alcohol-based hand rub was determined by MTT-assay. The order of cell viability was as follows: Ethanol for disinfection = ISODINE PALM > WELLUP > WELPAS (Fig. 7). The RhE exposed to ISODINE PALM was stained with povidone iodine, but the stain disappeared after the incubation and did not affect the MTT-assay.

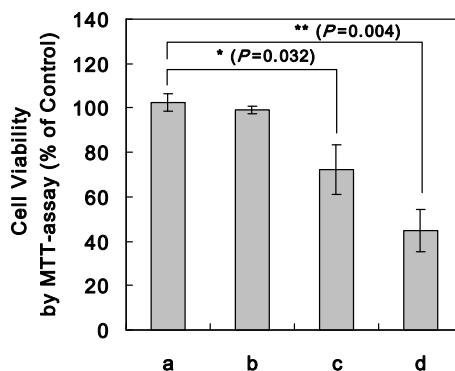


Fig. 7. Cell viability determined by MTT-assay; a: ethanol for disinfection, b: ISODINE PALM, c: WELLUP, d: WELPAS. The cell viability of RhE exposed to alcohol-based hand rub is expressed as a percentage relative to untreated one (negative control). Data are presented as means \pm SD ($n=3$). * $P < 0.05$ and ** $P < 0.01$ compared with ethanol for disinfection.

Comparative analysis between *in vivo* and *in vitro* experiments

The cell viabilities obtained from SMBM (this study) and the skin irritation index obtained from Draize rabbit tests (previous study: Tsuji et al., 1993) were examined. Fig. 8 shows a high correlation between the cell viability and skin irritation index.

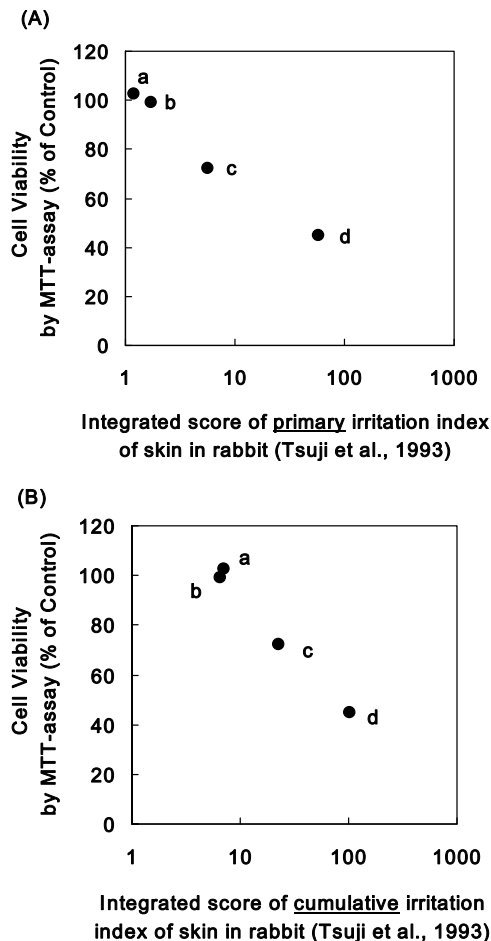


Fig. 8. Comparative analysis of *in vivo* and *in vitro* experiments. (A) Correlation of integrated score of primary irritation index of skin in rabbit and mean cell viability. (B) Correlation of integrated score of cumulative irritation index of skin in rabbit and mean cell viability.

7.1.3 Discussion

As already mentioned in Section 5.4, alcohols may enhance skin permeation, thus the irritancy of alcohol-based hand rubs containing antiseptics should be evaluated for the whole formulation, not for each component. In this study, a novel *in vitro* experimental method named SMBM was developed. SMBM mimics the actual usage of alcohol-based

hand rubs: putting on, spreading, rubbing into the skin and drying. As described in Section 4.3.2, cytotoxicity of ethanol in RhE is negligible for exposure shorter than 1 minute (Yamamoto et al., 2010). The results of the SMBM showed that the method can evaluate the overall irritation potential of the whole formulation of alcohol-based hand rubs containing antiseptics.

From the comparative analysis between *in vivo* and *in vitro* experiments, it was found that there was a high correlation between cell viability and skin irritation index. Therefore, SMBS is effective for quantitatively estimating the skin irritation potential of alcohol-based hand rubs containing antiseptics.

7.2 Evaluation of alcohol-based hand rubs containing cationic antiseptics by SMBM

7.2.1 Experimental

Alcohol-based hand rubs containing cationic antiseptics used in this study

The alcohol-based hand rubs used in this study are summarized in Table 5.

Code	Product Name	Antiseptics	Other components	Supplier*
A	Hibiscohol A	CHG 0.2%	Diisobutyl adipate, Allantoin, PEG glyceryl cocoate	Saraya
B	WELLUP	CHG 0.2%	Isopropyl myristate, 4 Non-disclosed components	Maruishi
C	WELLUP Hand Lotion 0.5%	CHG 0.5%	HM-HPMC**, 1,3-Butylene glycol, Glycyrrhetic acid, Diisopropyl adipate, Glycerine fatty acid ester, Buffering agent	Maruishi
D	WELPAS	BAC 0.2%	Propylene glycol, Isopropyl myristate, 4 Non-disclosed components	Maruishi
E	RABINET	BAC 0.2%	Urea, Glycerin, Tocopherol acetate, Allantoin, PCA ethyl cocoyl arginate	Kenei
F	Puremist	BAC 0.2%	Lipidure-PMB@***, Isopropyl myristate, Glycerin, 2 Non-disclosed components	Johnson & Johnson

Note: *Supplier: Saraya Co., Ltd., Maruishi Pharmaceutical Co., Ltd., Kenei Pharmaceutical Co., Ltd., Johnson & Johnson K.K. **HM-HPMC: Hydrophobically-modified hydroxypropyl methylcellulose. ***Lipidure-PMB@: Poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate).

Table 5. Alcohol-based hand rubs (76.9–81.4 vol% of ethanol) containing cationic antiseptics

Other materials and methods

Reconstructed human epidermis (RhE) and blowing equipment were prepared; and *in vitro* evaluation of skin irritancy and statistical analysis were carried out as previously described in Section 7.1.1.

7.2.2 Results

The mean cell viability of the 0.5% CHG formulation was slightly lower than that of 0.2% CHG formulations, but there were no significant differences in statistical analysis. On the other hand, the three 0.2% BAC formulations showed differences in cell viability (Fig. 9).

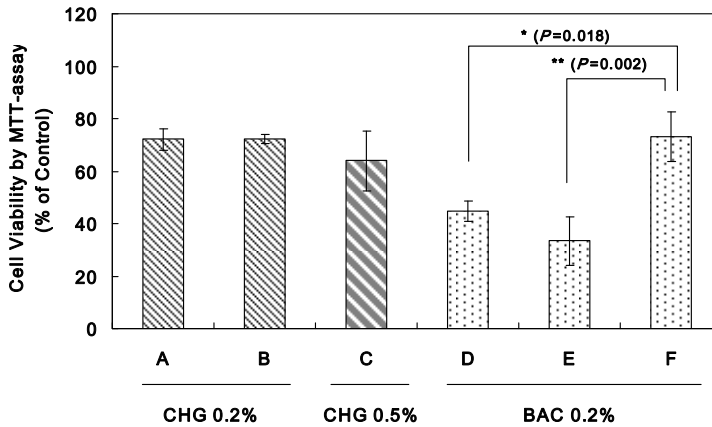


Fig. 9. Means \pm SD of cell viability of commercially available alcohol-based hand rubs determined by using SMBM

7.2.3 Discussion

Tested samples containing 0.2% or 0.5% CHG showed 64–72% cell viability, and therefore, their skin irritation potential was likely to be mild. The difference in CHG concentration did not significantly affect cell viability.

By contrast, in the case of BAC, the cell viability differed depending on formulation although the BAC concentration was the same. Of these, code F (Puremist) showed especially high cell viability (73% of cell viability). It was suggested that some components other than BAC may have reduced the skin irritation potential. Since isopropyl myristate and glycerin are also formulated in the other products (code D and E), they were unlikely to be the factor regulating the phenomenon. It is noteworthy that Lipidure-PMB® (MPC/BMA co-polymer) is contained in Puremist. As already mentioned in Section 6.2, MPC polymers stabilize the skin barrier. The results of this study suggest that the MPC polymers are possibly capable of reducing the cytotoxicity of alcohol-based hand rubs containing antiseptics.

8. Conclusion

In this review, the author summarized the structure and barrier function of the skin, the mechanism and evaluation methods of skin irritation, and the irritancy of alcohol-based hand rubs. It also described a novel *in vitro* evaluation method for assessing the skin irritation caused by alcohol-based hand rubs. The newly developed *in vitro* evaluation method “SMBM” has several advantages including 1) replacing animal experiments, 2)

enabling multiple substances to be tested at once, 3) easy quantitative estimation because it is based on simple cytotoxicity test. The author believes that this new approach is quite efficient and useful for developing less irritating alcohol-based hand rub products.

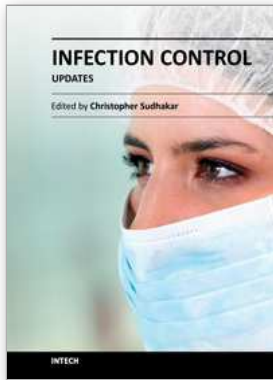
9. References

- Aly, R. & Maibach, H.I. (1979). Comparative study on the antimicrobial effect of 0.5% chlorhexidine gluconate and 70% isopropyl alcohol on the normal flora of hands. *Applied and environmental microbiology*, Vol. 37, pp. 610–613.
- Andoh, N.; Obi, N.; Miyazaki, T. & Ochiai, H. (2008). Antibacterial and skin protective effects of polyquaternium-51-containing ethanol, *Japanese Journal of Environmental Infections*, Vol. 23, pp. 35–40.
- Asselineau, D.; Bernhard, B.; Bailly, C. & Darmon, M. (1985). Epidermal morphogenesis and induction of the 67 kD keratin polypeptide by culture of human keratinocytes at the liquid-air interface, *Experimental cell research*, Vol. 159, pp. 536–539.
- Barel, A.O. & Clarys, P. (1995). Study of the stratum corneum barrier function by transepidermal water loss measurements: comparison between two commercial instruments: Evaporimeter and Tewameter, *Skin pharmacology*, Vol. 8, No. 4, pp. 186–195.
- Bell, E.; Ehrlich, H.; Buttle, D. & Nakatsuji, T. (1981). Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness, *Science*, Vol. 211, pp. 1052–1054.
- Boyce, J.M., Kelliher, S. & Vallande, N. (2000). Skin irritation and dryness associated with two hand-hygiene regimens: soap-and-water hand washing versus hand antiseptics with an alcoholic hand gel, *Infection control and hospital epidemiology*, Vol. 21, pp. 442–448.
- Boyce, J.M. & Pittet, D. (2002). Guideline for Hand Hygiene in Health-Care Settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HIPAC/SHEA/APIC/IDSA Hand Hygiene Task Force, *American journal of infection control*, Vol. 30, pp. S1–46.
- Cohen, J.C.; Hartman, D.G.; Garofalo, M.J.; Basehoar, A.; Raynor, B.; Ashbrenner, E. & Akin, F.J. (2009). Comparison of closed chamber and open chamber evaporimetry, *Skin research and technology*, Vol. 15, No. 1, pp. 51–54.
- de Haan, P.; Meester, H.H.M. & Bruynzeel, D.P. (1996). Irritancy of alcohol, In: *The Irritant Contact Dermatitis Syndrome*, Van der Valk, P.G. & Maibach, H.I. (Ed.), pp 65–70, CRC Press Inc., Boca Raton, FL.
- de Paepe, K.; Houben, E.; Adam, R.; Wiesemann, F. & Rogiers, V. (2005). Validation of the VapoMeter, a closed unventilated chamber system to assess transepidermal water loss vs. the open chamber Tewameter. *Skin research and technology*, Vol. 11, No. 1, pp. 61–69.
- Edwards, C.C. (1972). Hazardous substances. Proposed revision of test for primary skin irritants, *Federal Register*, Vol. 37, 27635–27636.
- Gallin, J.I.; Goldstein, I.M. & Snyderman, R. (Ed.). (1992). *Inflammation: Basic Principles and Clinical Correlates*, 2nd Ed., Ravan Press, New York, NY.
- Genno, M.; Yamamoto, R. & Kojima, H. (1998). Evaluation of a New Alternative to Primary Draize Skin Irritation Testing Using the EpiDerm™ Skin Model. *AATEX (Alternatives to animal testing and experimentation)*, Vol. 5, pp. 195–200.

- Green, H.; Kehinde, O. & Thomas, J. (1979). Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 76, No. 11, pp. 5665–5668.
- Hamasuna, R.; Takahashi, S.; Yamamoto, S.; Arakawa, S.; Yanaihara, H.; Ishikawa, S. & Matsumoto, T. (2011). Guideline for the prevention of health care-associated infection in urological practice in Japan. *International journal of urology*, Vol. 18, pp. 495–502.
- Harrington, C. & Walker, H. (1903). The germicidal action of alcohol. *Boston Medical and Surgical Journal*, Vol. 148, pp. 548–552.
- Iwasaki, Y. & Ishihara, K. (2005). Phosphorylcholine-containing polymers for biomedical applications. *Analytical and bioanalytical chemistry*, Vol. 381, No. 3, pp. 534–546.
- Jono, K.; Uemura, T.; Kuno, M. & Higashide, E. (1985). Bactericidal activity and killing rate of benzalkonium chloride and chlorhexidine gluconate, *YAKUGAKU ZASSHI (Journal of the Pharmaceutical Society of Japan)*, Vol. 105, pp. 751–759.
- Kim, D.D.; Kim, J.L. & Chien, Y.W. (1996). Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid, *Journal of pharmaceutical sciences*, Vol. 85, pp. 1191–1195.
- Kanekura, T.; Nagata, Y.; Miyoshi, H.; Ishihara, K.; Nakabayashi, N. & Kanzaki, T. (2002). Beneficial effects of synthetic phospholipid polymer, poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butyl methacrylate), on stratum corneum function, *Clinical and Experimental Dermatology*, Vol. 27, pp. 230–234.
- Kownatzki, E. (2003). Hand hygiene and skin health. *The Journal of hospital infection*, Vol. 55, pp. 239–245.
- Kramer, A.; Rudolph, P.; Kampf, G. & Pittet, D. (2002). Limited efficacy of alcohol-based hand gels, *Lancet*, Vol. 359, pp. 1489–1490.
- Larson, E.L.; Aiello, A.E.; Bastyr, J.; Lyle, C.; Stahl, J.; Cronquist, A.; Lai, L. & Della-Latta, P. (2001a). Assessment of two hand hygiene regimens for intensive care unit personnel, *Critical care medicine*, Vol. 29, pp. 944–951.
- Larson, E.L.; Aiello, A.E.; Heilman, J.M.; Lyle, C.T.; Cronquist, A.; Stahl, J.B. & Della-Latta, P. (2001b). Comparison of different regimens for surgical hand preparation, *Association of Operating Room Nurses Journal*, Vol. 73, pp. 412–418.
- Larson, E.L. & Morton, H.E. (1991). Alcohols, In: *Disinfection, sterilization and preservation, 4th ed.*, Block, S.S., (Ed.), pp. 191–203, Lea & Febiger, Philadelphia, PA.
- Lee, A-R.C. (2004). Phospholipid polymer, 2-methacryloyloxyethyl phosphorylcholine and its skin barrier function, *Archives of pharmacal research*, Vol. 27, pp. 1177–1182.
- Levin, C. & Maibach, H.I. (2004). Animal, human and *in vitro* test methods for predicting skin irritation, In: *Dermatotoxicology, Sixth Edition*, Zhai, H. & Maibach, H.I. (Ed.), pp. 678–690, CRC Press Inc., Boca Raton, FL.
- Li, L.N.; Margolis, L.B. & Hoffman, R.M. (1991). Skin toxicity determined *in vitro* by three-dimensional, native-state histoculture. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 88, pp. 1908–1912.
- Lowbury, E.J.; Lilly, H.A. & Ayliffe, G.A. (1974). Preoperative disinfection of surgeons' hands: use of alcoholic solutions and effects of gloves on skin flora. *British medical journal*, Vol. 4, pp. 369–372.
- Löffler, H.; Kampf, G.; Schmermund, D. & Maibach, H.I. (2007). How irritant is alcohol?, *The British journal of dermatology*, Vol. 157, pp. 74–81.

- Löffler, H. & Happle, R. (2003). Profile of irritant patch testing with detergents: sodium lauryl sulfate, sodium laureth sulfate and alkyl polyglucoside, *Contact Dermatitis*, Vol. 48, pp. 26–32.
- Lübbe, J.; Ruffieux, C.; van Melle, G. & Perrenoud, D. (2001). Irritancy of the skin disinfectant n-propanol, *Contact dermatitis*, Vol. 45, pp. 226–231.
- Merianos, J.J. (1991). Quaternary ammonium antimicrobial compounds. In: *Disinfection, sterilization, and preservation, 4th ed.*, Block, S.S. (Ed.), pp. 225–255, Lea & Febiger, Philadelphia, PA.
- Mezei, M.; Sager, R.W.; Stewart, W.D. & DeRuyter, A.L. (1966). Dermatitic effect of nonionic surfactants. I. Gross, microscopic, and metabolic changes in rabbit skin treated with nonionic surface-active agents, *Journal of Pharmaceutical Sciences*, Vol. 55, pp. 584–590.
- Morota, K.; Morikawa, N.; Morita, S.; Kojima, H. & Konishi, H. (1999). Alternative to primary Draize skin irritation test using cultured human skin model: Comparison of six endpoints, *AATEX (Alternatives to animal testing and experimentation)*, Vol. 6, pp. 41–51.
- Murphy, J.C.; Watson, E.S.; Wirth, P.W.; Skierkowski, P.; Folk, R.M. & Peck, G. (1979). Cutaneous irritation in the topical application of 30 antineoplastic agents to New Zealand white rabbits, *Toxicology*, Vol. 14, pp. 117–130.
- Nagasawa, M.; Hayashi, H. & Nakayoshi, T. (2002). *In vitro* evaluation of skin sensitivity of povidone-iodine and other antiseptics using a three-dimensional human skin model, *Dermatology*, Vol. 204, pp. 109–113.
- National Academy of Sciences and Committee for the Revision of NAS Publication 1138. (1977). Principles and procedures for evaluating the toxicity of household substances, pp. 23–59, National Academy of Sciences, Washington DC.
- Nixon, G.A.; Tyson, C.A. & Wertz, W.C. (1975). Interspecies comparisons of skin irritancy, *Toxicology and Applied Pharmacology*, Vol. 31, pp. 481–490.
- Newman, J.L. & Seitz, J.C. (1990). Intermittent use of an antimicrobial hand gel for reducing soap-induced irritation of health care personnel, *American journal of infection control*, Vol. 18, pp. 194–200.
- Ojajärvi, J. (1991). Handwashing in Finland, *Journal of Hospital Infection*, Vol. 18(suppl B), pp. 35–40.
- Patil, S.M.; Patrick, E. & Maibach, H.I. (1996). Animal, human and *in vitro* test methods for predicting skin irritation, In: *Dermatotoxicology*, Fifth Edition, Marzulli, F.N. & Maibach, H.I. (Ed.), pp. 411–436, Taylor and Francis, Washington DC.
- Patil, S.M.; Patrick, E. & Maibach, H.I. (1998). Animal, human and *in vitro* test methods for predicting skin irritation, In: *Dermatotoxicology methods: the laboratory worker's vade mecum*, Marzulli, F.N. & Maibach, H.I. (Ed.), pp. 89–104, Taylor and Francis, Washington DC.
- Patrick, E. & Maibach, H.I. (1989). Comparison of the time course, dose response and mediators of chemically induced skin irritation in three species. In: *Current Topics in Contact Dermatitis*, Frosch, P., Doooms-Goossens, A., Lachapelle, J.M., Rrcroft, R.J.G. & Scheper, R.J. (Ed.), pp. 399–402, Springer-Verlag, New York.
- Price, P.B. (1938). New studies in surgical bacteriology and surgical technic. *The Journal of the American Medical Association*, Vol. 111, pp. 1993–1996.
- Rotter, M. (1999). Hand washing and hand disinfection. In: *Hospital epidemiology and infection control, 2nd ed.*, Mayhall, C.G., (Ed.) , pp. 1339–1355, Lippincott Williams & Wilkins, Philadelphia, PA.

- Shillaker, R.O.; Bell, G.M.; Hodgson, J.T. & Padgham, M.D. (1989). Guinea pig maximization test for skin sensitisation: The use of fewer test animals, *Archives of Toxicology*, Vol. 63, pp. 283–288.
- Shimizu, M.; Okuzumi, K.; Yoneyama, A.; Kunisada, T.; Araake, M.; Ogawa, H. & Kimura, S. (2002). *In vitro* antiseptic susceptibility of clinical isolates from nosocomial infections, *Dermatology*, Vol. 204, pp. 21–27.
- Skog, E. (1960). Primary irritant and allergic eczematous reactions in patients with different dermatoses, *Acta dermato-venereologica*, Vol. 40, pp. 307–312.
- Triglia, D.; Sherard Braa, S.; Yonan, C. & Naughton, G.K. (1991). Cytotoxicity testing using neutral red and MTT assays on a three-dimensional human skin substrate. *Toxicology in vitro*, Vol. 5, pp. 573–578.
- Tsuboi, R.; Arai, K.; Sumida, H.; Nishio, M.; Hasebe, K.; Hioki, Y. & Okuda, M. (2006). Efficacy of an alcohol-based hand rub containing synthetic pseudo-ceramide for reducing skin roughness of the hand, *Japanese Journal of Environmental Infections*, Vol. 21, No. 2, pp. 73–80.
- Tsuji, A.; Nakayoshi, T.; Sannomiya, F.; Yashiro, J. & Goto, S. (1993). Rabbit skin irritation of alcoholic solutions for hand disinfection, *Japanese Journal of Environmental Infections*, Vol. 8, No. 2, pp. 33–41.
- Tsuji, A.; Sannomiya, F.; Yashiro, J.; Nakajima, S. & Goto, S. (1996). Skin irritation study of antiseptics with rabbit, *Japanese Journal of Environmental Infections*, Vol. 11, No. 3, pp. 207–220.
- Warner, R.R.; Stone, K.J. & Boissy Y.L. (2003). Hydration disrupts human stratum corneum ultrastructure, *The Journal of investigative dermatology*, Vol. 120, pp. 275–284.
- Watkinson, R.M.; Herkenne, C.; Guy, R.H.; Hadgraft, J.; Oliveira, G. & Lane, M.E. (2009). Influence of ethanol on the solubility, ionization and permeation characteristics of ibuprofen in silicone and human skin, *Skin pharmacology and physiology*, Vol. 22, pp. 15–21.
- Weil, C.S. & Scala, R.A. (1971). Study of intra- and interlaboratory variability in the results of rabbit eye and skin irritation tests. *Toxicology and Applied Pharmacology*, Vol. 19, pp. 276–360.
- Winnefeld, M.; Richard, M.A.; Drancourt, M. & Grob, J.J. (2000). Skin tolerance and effectiveness of two hand decontamination procedures in everyday hospital use. *The British journal of dermatology*, Vol. 143, pp. 546–550.
- World Health Organization (2009). WHO Guidelines on Hand Hygiene in Health Care.
- Yamamoto, N.; Miyamoto, K. & Katoh, M. (2010). Development of alternative to animal experiment in evaluation of skin irritation caused by alcohol-based hand rubs. *YAKUGAKU ZASSHI (Journal of the Pharmaceutical Society of Japan)*, Vol. 130, pp. 1069–1073.
- Yamamoto, N.; Shuto, K.; Yamagishi, T. & Nakamoto, Y. (2007). Self-assembled mosaic lamellar structures in hydrophobic phospholipid polymer films. *KOBUNSHI RONBUNSHU (Japanese Journal of Polymer Science and Technology)*, Vol. 64, pp. 115–118.



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Health care associated infection is coupled with significant morbidity and mortality. Prevention and control of infection is indispensable part of health care delivery system. Knowledge of Preventing HAI can help health care providers to make informed and therapeutic decisions thereby prevent or reduce these infections. Infection control is continuously evolving science that is constantly being updated and enhanced. The book will be very useful for all health care professionals to combat with health care associated infections.

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