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An Assessment of the Endotoxin Contents of Natural Rubber Latex Medical Gloves

A. IKRAM* AND M.Y. AMIR-HASHIM*#

The endotoxin levels of Malaysian Natural Rubber (NR) latex medical gloves were determined by a kinetic turbidimetric Limulus Amoebocyte Lysate (LAL) assay. The samples evaluated were 39 pairs of sterilised surgical gloves from 10 commercially-available brands and 21 pairs of non-sterilised examination gloves from 8 brands. The endotoxin concentrations ranged from 3.2 to 114.1 EU/glove pair for surgical gloves and from <8.4 to 9,632 EU/glove pair for the examination gloves. In the case of surgical gloves, 72% had endotoxin activity below the specified FDA standard of 20 EU/device, while in the case of the non-sterilised examination gloves, only 38% had values below the specified level. There was no correlation between endotoxin concentrations and the presence of glove powders in the present study.

Key words: NR latex gloves; surgical gloves; examination gloves; Limulus Amoebocyte Lysate (LAL); lipopolysaccharides (LPS); Gram-negative bacteria; Control Standard Endotoxin (CSE)

Endotoxin is a lipopolysaccharide (LPS) component of the outer cell wall membrane of the environment-ubiquitous Gram-negative bacteria that is known to produce a variety of inflammatory responses in human and animal subjects when they find their way into the mammalian blood system in clinically-relevant amounts[1–9]. The host symptoms range from fever and septic shock to hypotension, adult respiratory distress syndrome, airway inflammation and disseminated intravascular coagulation. The potential to induce fever has led endotoxins to be also referred to as pyrogens. LPS stimulates ("activates") alveolar macrophages and respiratory epithelial tissue to release proinflammatory cytokines (chemoattractants that initiate an inflammatory cascade) such as interleukin (IL)-1β, IL-6, IL-10 and the tumour necrosis factor α (TNF-α), some of which travels through blood to the hypothalamus, the body's thermoregulatory centre in the brain[10]. Endotoxins are fragmented remains of bacteria that are bioactive and may adhere on implants/medical devices even after sterilisation. Thus, endotoxin contamination control is important in manufacturing human and animal drug products, biologics and medical devices, and in haemodialysis therapy.

Endotoxin was previously shown to be a significant contaminant on natural rubber (NR) latex gloves[11–17]. The detection of endotoxin is of significance since commercially-available medical devices need to have low endotoxin levels before they can be approved for sale

*Rubber Research Institute of Malaysia, Malaysian Rubber Board, P.O. Box 10150, 50908 Kuala Lumpur.
Corresponding author (e-mail: amir@lgm.gov.my)

by the regulatory authorities. In this study, we evaluated the endotoxin content of some commercially-available NR surgical and examination gloves by the sensitive Limulus amoebocyte lysate (LAL) assay.

MATERIALS AND METHODS

Gloves

Thirty-nine pairs of commercially-available sterile NR latex surgical gloves from 10 brands and 21 pairs of non-sterile examination gloves from 8 brands were used. Except for brand A that had gloves from 2 lots, all other brands were from the same lot.

Glassware

All glassware was depyrogenated by dry heat (180ºC) for 4 h prior to use.

Water

Sterile distilled LAL Reagent Water (LRW, <0.001 EU/mL; Associates of Cape Cod, Inc., Falmouth MA) was used as negative controls, for reconstituting the lyophilised reagent and the control endotoxin standard.

Extraction

The outer surface of the glove samples was extracted for assay using the procedure outlined in *BS EN 455-3: 2000*[18]. Gloves were inverted "outside in" using sterile glass rods where necessary in a laminar flow chamber. Sterile non-pyrogenic water for injection (WFI) was pipetted into the inner glove (20 mL), the gloves clamped to produce a watertight seal, and squeezed horizontally before being shaken on a horizontal platform at 37–40ºC for 1 h (150 r.p.m.). The contents were then centrifuged at 2,000g for 15 mins to clarify the extract. The extracts were subsampled for pH checks and where necessary, the pHs adjusted to within the range of 7.0 – 8.0 using endotoxin-free 0.1M NaOH or 0.1M HCl.

LAL reagent

The LAL reagent (Pyrotell-T®; Associates of Cape Cod, Inc, Falmouth, MA) was reconstituted immediately before use with LRW, and transferred to each microplate well as rapidly as possible with a repetitive pipette.

Control standard endotoxin

Control standard endotoxin (CSE) was used to construct standard curves, validate product and to prepare inhibition controls. For each assay, standard curves were generated over the concentration ranges 0.03125-1.000 endotoxin units (EU)/mL using a reference CSE (*Escherichia coli* O113:H10; Associates of Cape Cod, Inc, Falmouth, MA), to give 6 points covering a 2-log range with assay sensitivity (λ) = 0.03 and the maximum valid dilution (MVD) = 32X.

Endotoxin assay

Endotoxin concentration was determined by a kinetic turbidimetric Limulus amoebocyte lysate (LAL) assay. A series of endotoxin standards and sample extracts, as well as the spiked sample, in 2-fold serial dilutions were prepared in duplicate wells of pyrogen-free microtitre plates using a 6-channel micropipetter (Nunc A/S, Denmark). The spiked dilutions were included to test for performance of inhibition or enhancement.

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The routine test protocol includes LRW blanks as negative controls and a known amount of endotoxin standard as positive controls. The samples were then reacted with the LAL reagent in each well. The assay plate was placed in a ELx808i microplate reader (Bio-Tek Instruments, Inc., Vermont, USA) and the assay was allowed to proceed at 37±1ºC for 1h. Spectrophotometric measurements at 340 nm were taken at every 20 s interval, and the data analyzed using KC4™ Software (Biotek Instruments, Inc.) where the sample concentrations were computed at minimum acceptable standard curve values of $r^2 = 0.998$. The kinetic software calculates the "onset time" for the sample in each well to reach a specified optical density value ("onset OD"), generates the standard curve parameters (slope, intercept, correlation coefficient) and calculates the endotoxin concentrations in the unknown samples. The results are reported as endotoxin potency (EU/per mL) and tabulated as EU/pair gloves.

RESULTS

The endotoxin levels varied over a wide range for the non-sterile examination gloves (from <8.4 to 9,632 EU/glove pair) but were narrower for the surgical gloves (3.2 to 114.1 EU/glove pair) (*Table 1*). Of the 39 sterile surgical glove pairs sampled, 3 (Brand J, Biogel-coated) did not yield results but 26 of the remaining 36 pairs (72%) had detectable levels of <20 EU/pair. As a comparison, 8 out of the 21 pairs (38%) of the examination gloves examined showed levels of <20 EU/pair. The determination for Brand J gloves was not possible since the extracts inhibited the LAL assay.

For both the surgical and examination gloves, there appeared to be no correlation between powdered or powder-free gloves and their endotoxin contents.

DISCUSSION

Standards proposed by the US Food and Drug Administration (FDA) for medical devices limit the endotoxin level to less than 20 EU/device[19], and thus surgical gloves have to be made sufficiently clean to meet the specifications. This is the level required of medical devices that come in contact with blood or lymph circulating in a patient, although the device limit for cerebrospinal fluid is more stringent (2.15 EU). In our previous work with non-sterile examination gloves prepared under sufficiently clean conditions[16], we have shown that bacterial endotoxin activity may range from <50-183 EU/glove. In the present study however, the levels for examination gloves were much wider and ranged to a high of 4,816 EU/glove (*Table 1*). This was not unexpected, since examination gloves were not sterile when packed. In fact, Thorne *et al*.[17] tested eight types of medical examination gloves and found endotoxin contents ranging from below detection (<1.5 EU) to 5 810 EU. Although glove powders can act as a vehicle for latex allergens and endotoxins, and was the basis of selection for some of the earlier experiments[13,14], there appeared to be no correlation between the endotoxin concentrations determined and the presence of glove powders in the current study.

It can be seen that most of the sterile surgical gloves used in the present study was generally clean, with values that can be categorised to be in the minor to moderate contamination range. Asplund Peiro *et al*.[12] had previously tested 16 batches of sterile surgical gloves and categorised glove contamination as minor (0.2-9.0 EU/glove), moderate (15-31 EU/glove) and heavy (138-1 071 EU/glove). Grotsch *et al*.[20] also determined the levels of endotoxin on sterile surgical gloves from five manufacturers and showed that for all gloves, the outer surface had very low or undetectable endotoxin contamination.

TABLE 1. GLOVE ENDOTOXIN CONTENT OF NR LATEX SURGICAL AND EXAMINATION GLOVES AS DETERMINED BY THE LAL ASSAY*

Glove #	Endotoxin content, EU/pair glove			
	Surgical gloves	Brand	Examination gloves	Brand
1	7.6	A (P)lot 1	20.8	K (P)
2	8.0	A (P)lot 1	13.9	K (P)
3	10.8	A (P)lot 1	16.5	K (P)
4	11.8	A (P)lot 1	13.5	L(P)
5	22.8	A (P)lot 2	8.6	L (P)
6	9.0	A (P)lot 2	11.1	L (P)
7	10.5	A (P)lot 1	2,692	M (PF)
8	3.2	A (P)lot 1	3,578	M (PF)
9	9.5	A (P)lot 1	1,103	M (PF)
10	6.9	A (P)lot 1	9,632	N (PF)
11	4.3	B (PF)	<8.4	N (PF)
12	5.4	B (PF)	11.4	N (PF)
13	6.0	B (PF)	18.1	O (P)
14	11.2	C (P)	130.8	O (P)
15	11.9	C (P)	21.2	O (P)
16	10.9	C (P)	515.7	P (P)
17	4.3	D (P)	743.7	P (P)
18	24.7	D (P)	292.6	Q (P)
19	23.3	D (P)	152.3	Q (P)
20	23.6	D (P)	111.0	Q (P)
21	9.7	E (LP)	98.2	Q (P)
22	21.3	E (LP)		
23	4.8	E (LP)		
24	43.0	F (PF)		
25	114.1	F (PF)		
26	41.4	F (PF)		
27	7.0	G (P)		
28	10.7	G (P)		
29	26.4	G (P)		
30	14.4	H (P)		
31	26.8	H (P)		
32	13.4	H (P)		
33	<16.8	I (PF)		
34	<16.8	I (PF)		
35	9.3	I (PF)		
36	18.4	I (PF)		
37	-	J (PF-B)		
38	-	J (PF-B)		
39	-	J (PF-B)		

*Assayed in duplicate. P=powdered; LP=lightly-powdered; PF=powder-free; PF-B=Powder-free, Biogel coated.

However, the inside surface of the gloves varied, three showed contamination ranging from 160 EU/glove to greater than 2 560 EU/glove. During manufacture, the inside surfaces of gloves were previously the outside prior to stripping and were therefore most exposed to environmental contamination. Holmdahl and Chegini[14] tested 14 different brands of sterile surgical gloves (nine powdered, five powder-free) and found that the amount of endotoxin on the outer surface of a single surgical glove from four of the powder-free brands was low, and one brand had levels high enough to cause potentially adverse events post-operatively. Of the nine powdered gloves, two carried four to eight times the normal quantity. Brock Williams and Halsey[13] tested 19 NR surgical and examination gloves and two (vinyl and neoprene) surgical gloves and found that the examination gloves averaged 4 040 EU/g glove (=30 704 EU/glove for a 7.6 g glove) and surgical gloves averaged 30 EU/g glove (=228 EU/glove). Such large amounts of bacterial LPS were much higher than any reported in the literature. In another study using the LAL kinetic chromogenic assay on five surgical gloves and two Foley catheters, water extracts from one glove and one catheter showed strong LAL activities equal to 3.2 and 13.6 ng/mL, respectively[15]. Assuming 1 ng/mL is equivalent to 5 EU (depending on the reference endotoxin)[9], this works out to 16 and 68 EU/mL respectively, for the glove and catheter type examined.

The level of endotoxins present on the device could influence the outcome of inserting a medical device into the body and complications that could arise from contaminated surgery. This possibility is the reason for the concern with endotoxin contamination control and why pyrogenicity testing has been performed on medical devices ever since guidelines were drawn up for their biological testing[9]. The host response to endotoxin is a highly complex field, and humans develop a reaction to endotoxin when injected at 4 ng/kg body weight[9].

Reducing the likelihood of bacterial contamination reduces both microorganisms and pyrogens. In the present study, the better results obtained for the new generation of sterile surgical gloves compared to values reported in the literature were due to improved manufacturing technologies and better environmental control of particulate and biological contamination. Clean zones help prevent product contamination, and surgical gloves are packed in cleanrooms with regularly cleaned and sanitised working surfaces, equipment as well as gowned personnel. The variation in endotoxin activity between brands probably reflects this aspect of environmental control.

Gram-negative bacteria are found as normal microflora of soils, water and living organisms, and endotoxins are thus ubiquitous in both outdoor and indoor environments. High occupational airborne-endotoxin exposure is already known in agricultural and related industries (crop harvesting, cotton and vegetable processing, livestock barns, grain handling, slaughter houses), textiles, pulp and paper processing, sawmills, composting, sewage and domestic waste handling, wastewater treatment, fibreglass manufacturing and metal machining environments[21]. Indeed, a number of epidemiologic studies have revealed the association between endotoxin exposure and respiratory symptoms or pulmonary function decline[17,22]. However, the sampling of airborne bacteria and endotoxins in highly contaminated environments can be problematic. In the rubber industry, there have been no studies for endotoxin exposure assessment, to identify specific locations and tasks associated with high exposure to endotoxins or of endotoxin contamination related to product manufacture, as is common in the food, beverage and pharmaceutical industries.

CONCLUSION

We observed that the endotoxin contamination of commercially-available sterile NR latex surgical gloves from the same lots were quite variable between brands in having low to intermediate concentrations of endotoxin activity. With some exceptions, most of these surgical gloves had levels below the FDA specified standards of <20 EU/device. In contrast, most of the non-sterile NR latex examination gloves were highly contaminated, with a higher percentage exceeding the specified detectable level.

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