Standardization of needlestick injury and evaluation of a novel virus-inhibiting protective glove

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Summary Rubber surgical gloves worn as a barrier to prevent contamination from body fluids offer relative protection against contamination through direct percutaneous injuries involving needles, scalpel blades or bone fragments. To determine the main experimental parameters influencing the volume of blood transmitted by a hollow-bore needle (worst case scenario) during an accidental puncture, we designed an automatic puncture apparatus. Herpes simplex type 1 virus (HSV1), a model for enveloped viruses, was used as a 'marker' in an in-vitro gelatine model. Of the experimental parameters studied, the most critical influences were found to be needle diameter and puncture depth, whereas puncture speed, puncture angle and glove-stretching feature appeared to be less influential. A single glove reduced the volume of blood transferred by 52% compared with no glove, but double gloving offered no additional protection against hollow-bore needle punctures. Using 'standardized' puncture conditions, the virus-inhibiting surgical glove G-VIR® elicited an 81% reduction in the amount of HSV1 transmitted as compared with single or double latex glove systems.

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Introduction

Healthcare professionals have major concerns about occupational exposure to blood from viruses-infected patients. Currently, hepatitis B virus (HBV) can be prevented by vaccination whereas only post-exposure prophylaxis is available for human immunodeficiency virus (HIV) and no immediate prophylaxis is available for hepatitis C virus (HCV). Among all blood exposure accidents reported, sharp (needlestick) injuries are associated with the highest contamination risk, estimated at 0.5–3% for HCV and 0.3% for HIV.1,2

Hand protection is recommended to prevent exposure to blood and bodily fluids, but the safety offered by single- or even double-gloving systems in case of needlestick injuries has been often questioned. Rubber gloves do not protect against needles penetrating the skin and the efficacy of such ‘passive’ layers in preventing virus transmission relies on a cleaning effect of the puncturing item external surface. This is particularly true when items of simple geometry such as suture needles are considered, but this ‘wiping effect’ becomes inefficient in the case of deep punctures involving hollow-bore needles or other sharp objects such as scalpel blades, porous bone fragments, etc.3 To achieve a higher level of protection, the G-VIR® glove was designed with a layer incorporating a liquid disinfectant in drop-like compartments sandwiched between two mechanical layers. In the event of accidental puncture or laceration, release of this liquid provides protection at the site of damage by significantly reducing the amount of transmitted viruses.4,5

The basic transmission risk factor is the quantity of inoculated viruses, directly linked to the patient viral load and to the volume accidentally inoculated.6

To establish a reproducible test simulating a hollow-bore needlestick accident with accurate control of experimental parameters, we designed an automated puncture apparatus using in-vitro quantitative detection of transmitted infectious viruses. We report the results of an initial series of experiments, using type 1 herpes simplex virus (HSV1) as a ‘marker’, to study the main mechanical parameters influencing the volume of blood transferred, including puncture depth, needle diameter, puncture speed and angle, glove thickness and glove stretching.

Defined ‘standardized’ experimental conditions were then used to assess the protective feature of the G-VIR® glove in comparison with ‘reference’ gloves using HSV1 as a model for enveloped viruses such as HIV and HCV.5

Methods

Gloves

Three types of gloves were used: the G-VIR® glove — a synthetic, powder-free surgical glove (Hutchinson Santé, France) containing a liquid disinfectant (quaternary ammonium salts and chlorhexidine) evenly distributed in the thickness in the form of microdroplets. The resulting thickness is 500 μm; and two references: ‘blank’ gloves supplied by Hutchinson Santé, similar to G-VIR® but without disinfectant inside, and standard latex surgical gloves (Dermotact®, Wuhrlin Soplamed, Courbevoie, France) used either as single (250 μm) or double (500 μm) thickness.

Cell culture

Vero cells (ECACC no. 84113001, ATCC, UK) were cultured in Dulbecco’s modified Eagle’s medium (Sigma, Saint Quentin Fallavier, France), supplemented with L-glutamine, antibiotics and 10% fetal calf serum (Gibco, Paisley UK). Cell monolayers were grown in an incubator (37 °C, 5% CO2). During passage of primary cultures, cells were detached using 0.1% trypsin/EDTA solution. Medium was changed every three days.

Virus strain

The HSV1 tested was the Bey strain, cultured on Vero cells. Infected cell monolayers were harvested when viral cytopathic effects were present on all cells. After three freeze-thawed cycles, the virus was homogenized, and stored in aliquots with its cellular debris in growth medium at −74 °C until use. The titre of infectious material determined by a standard plaque assay was 107 plaque-forming units (pfu)/mL.7 In all experiments, the viral solution was used at 106 pfu/mL prepared by 10-fold dilution in defibrinated sheep blood (AES Chemunex, France). The viral titre was checked at the beginning and at the end of the experiments and showed good stability. The same viral stock was used for all experiments.

Automated puncture apparatus

The apparatus consists of a pneumatic actuator supporting the needle and the syringe mounted on a vertical sliding guide, which allows accurate setting of the puncture depth. Air pressure was controlled with a manometer, the flow being varied to modify the puncture speed.
Square samples (8 × 8 cm) were cut from the back of the gloves and fixed to a rigid frame to control stretching. The puncture angle between the needle and the glove sample was set either at 90° or at 45° by placing the sample horizontally or on a rack at 45° over the collecting medium, respectively.

All punctures were performed with a reproducible resident time of the needle in the collecting medium of <0.5 s.

**Puncture test**

Hollow-bore needles (Microlance, Becton Dickinson, Fraga (Huesca), Spain) and catheters (Jelco, Medex, Haslingden, UK) ranging from 25G to 16G were attached to a 1 mL disposable syringe (Terumo Europe, Leuven, Belgium) and inserted 2 cm into the viral suspension. Blood was aspirated through the needle into the syringe and ejected to ensure the presence of a blood droplet at the needle tip, then the syringe was attached onto the puncture apparatus.

Glove samples were punctured at a given speed, depth and angle with no force exerted on the plunger of the syringe. The transmitted volume of blood was collected in a microtube (Eppendorf AG, Hamburg, Germany) filling with 1.2 mL collecting medium (DMEM). The collecting medium was slightly jellified with 4%-by-weight gelatine (LPB150, Rousselot SAS, Courbevoie, France) to mimic skin effect and to avoid capillarity passive transfer.

After the puncture, the collecting medium was liquefied at 37°C, then carefully overlaid on a confluent cell monolayer cultured in a 60 mm tissue culture dish (Becton Dickinson). After 2 h incubation (37°C, 5% CO₂) culture medium was supplemented with 1% human serum (Sigma) and after further incubation for 3 days plaques were counted visually.

A series of 15 punctures (one per microtube) was performed for each experimental condition studied; a new needle was used for each puncture.

**Volume vs pfu calibration curve**

A calibration curve taking into account the cellular response in the presence of gelatine and of blood was constructed to set the equivalence between the number of plaques counted and the corresponding transmitted volume.

Exactly 1 μL jellified collecting medium containing 0.2 μL of a viral suspension in blood was deposited onto a cell monolayer for plaque counting with a range of dilutions of the initial viral suspension in blood (titres from 10⁵ to 5 × 10⁶ pfu/mL). The number of plaques counted was then reported versus the number of viruses deposited.

**Data analyses**

Three separate experiments were performed for each parameter studied. Means of the 45 collected data for each experimental condition were calculated and expressed with a 99.5% confidence interval using Student’s t-test. To define parameters significantly affecting blood volume, differences between means were assessed by analysis of variance (ANOVA) using Statgraphics Plus (v5.1, Manugistics, Inc., Rockville, USA) software; P < 0.05 was considered significant. When of particular interest, fit of experimental results was performed with a regression analysis.

**Results**

**Protocol development**

Initial experiments to determine the influence of the main mechanical parameters on the volume of blood transmitted were realized with widely used 22G hollow-bore injection needles. Unless otherwise stated, punctures were performed through a single glove layer.

*Calibration curve*. Linear regression analysis of the number of plaques counted vs the number of viruses deposited showed good fit to a line of equation $y = 0.78x + 20.6$ (data not shown, $R^2 = 0.96$, $P = 0.022$). Since all the punctures were performed with the same viral stock, this calibration curve was used in all experiments to determine blood volumes as a function of the titre of the viral suspension and the number of plaques counted.

*Puncture depth* (Figure 1a). Transferred blood volume increased significantly with the puncture depth ($P < 0.001$), from 0.07 ± 0.02 μL (at 3 mm) to 0.24 ± 0.04 μL (at 15 mm). In the range considered, the volume variation was linear ($R^2 = 0.97$, $P = 0.0022$). A depth of 6 mm was considered relevant for injuries involving deep puncture and was defined as the standard.

*Needle size* (Figure 1b). The volume of blood transferred increased with the needle external diameter, and with the needle bore ($P < 0.001$), varying between 0.048 ± 0.01 μL (25G) and 0.47 ± 0.07 μL (16G). The results could be fitted to a power equation giving a square exponent ($R^2 = 0.97$, $P = 0.0027$). 22G was defined as the standard.

*Puncture speed*. No effect was observed on the volume of blood transferred ($P = 0.12$) between
15 cm/s (0.12 ± 0.03 µL) and 50 cm/s (0.14 ± 0.04 µL). The standard was defined at 15 cm/s as it corresponded to common hand moves.

Puncture angle. Punctures were performed with the open part of the needle tip turned on the side. The transmitted blood volume appeared to remain unchanged (P = 0.143) when setting the puncture angle either at 90° (0.15 ± 0.03 µL) or at 45° (0.17 ± 0.04 µL). This may be quite surprising since the needle is not an axis-symmetrical object, but only two specific configurations were studied in the range of possibilities. As we observed no difference, for practical reasons, the standard was defined at 90°.

Glove-stretching feature. Wiping of the external needle surface may depend on glove stretching, but a range of biaxial stretch ratio between 0 and 20% showed no significant effect on the volume of blood transferred (0.14 ± 0.03 µL without glove tension, 0.14 ± 0.04 µL for 20% stretch; P = 0.51). To remain within expected in-use conditions, punctures were studied on gloves stretched by 10%.

In the above-defined standard conditions — puncture speed (15 cm/s), puncture angle (90°), puncture depth (6 mm), needle diameter (22G), glove tension (10%) — to simulate needlestick injury, punctures through a single rubber glove with an HSV1-virus contaminated blood (1.3 × 10^6 pfu/mL), 15 cm/s puncture speed, 90° puncture angle, 10% glove tension. The bold line in (b) corresponds to a fit with a power equation of y = 0.7x^{2.09} (regression analysis).

Figure 1  Impact of (a) puncture depth and (b) needle diameter on transmitted blood volume. Transferred volumes, expressed in microlitres, were determined with puncture depths into jellified collecting media ranging from 3 to 15 mm using a 22G needle (a), or a puncture depth of 6 mm with hollow-bore needles of inner diameter ranging from 0.3 (25G) to 0.85 (16G) mm (b). The needle bores were determined using a binocular microscope. All determinations (N = 45 for each experimental condition) were performed through a single latex glove with HSV1-virus contaminated blood (1.3 × 10^6 pfu/mL), 15 cm/s puncture speed, 90° puncture angle, 10% glove tension. The bold line in (b) corresponds to a fit with a power equation of y = 0.7x^{2.09} (regression analysis).
0.12 ± 0.03 μL (P < 0.001) by a single rubber surgical glove. However, doubling the mechanical barrier thickness by superposition of two standard gloves (representing the recommended double-gloving practice) offered no additional reduction (0.12 ± 0.03 μL, P = 0.93). Therefore the mechanical wiping of the needle seems already maximized by one glove. Furthermore, the inner volume of the needle is likely to be unaffected by this wiping since the amount transferred remains large and constant for either one or two gloves. Similar results were observed with the 'blank' glove, equivalent in thickness to two gloves but in one material (0.12 ± 0.03 μL). Therefore the single latex glove was used as the reference for the following experiments on the G-VIR® glove.

Protective effect of G-VIR® on transmitted viral load during needlestick injury

When the viral titrations were assessed after punctures with an HSV1 titre of 1.2 × 10^6 pfu/mL, the G-VIR® glove caused an 81% reduction in the transmission of infectious viruses from 122 ± 23 pfu and 121 ± 27 pfu with the reference latex and 'blank' gloves, respectively, to 19 ± 6 pfu (P < 0.001), a difference which can only be attributed to a virucidal effect induced by the disinfecting agent in the G-VIR®. This effect results from a short contact time between the viruses and the disinfectant. Complementary experiments varying this contact time between 30 s (minimum time needed to liquefy the gelatine) and 10 min showed no difference (P = 0.127) in the viral count, showing the activity of the disinfectant is rapidly quenched by massive dilution (106-fold as determined by capillary electrophoresis) when the needle arrives in the collecting medium.

When experiments were performed at 22 or 37 °C, temperature had no effect using either the latex gloves (126 ± 12 pfu at 22 °C, 118 ± 12 pfu at 37 °C; P = 0.056) or the G-VIR® gloves (23 ± 6 pfu at 22 °C, 24 ± 6 pfu at 37 °C; P = 0.142).

The protective effect was also assessed as a function of the needle diameter (Figure 2). In the range considered, the G-VIR® glove resulted in a significant decrease (from 74% for 16G to 92% for 25G) of the amount of viral particles transmitted as compared to one latex glove (P < 0.001 for all needle sizes).

Discussion

We describe the first use of an automatic apparatus to study the influence of the mechanical parameters of puncture and glove on the volume of blood transferred during a simulated needlestick accident with hollow-bore needles.

The use of an automated apparatus ensures good reproducibility and the ability to control experimental puncture parameters. Moreover,
our model was complemented by using a jellified collecting medium to simulate as closely as possible the mechanical effect of the skin. Indeed, the use of a gelatine medium provides advantages of a collagen viscoelastic structure close to that of skin, and avoids volume uncertainty due to capillary phenomena encountered in liquid or absorbent media.8

For the standard protection offered by latex gloves, we found that the amount of transmitted blood is directly linked to the internal needle diameter (bore) and to the penetration depth. In contrast, puncture speed, puncture angle and glove tension had little effect. In our defined standard conditions the volume of blood transferred through one latex glove was 0.14 ± 0.03 μL. Several studies in the literature have reported blood volumes from the same gauge (22G) between 0.06 and 1.4 μL.9 This wide range of results may be the consequence of differences in the models and experimental techniques involved. But more importantly, previous results were associated with large intra-experimental variability probably due to the punctures being performed manually.

We have shown that a single latex glove decreases the volume of transmitted blood after hollow needle puncture by ~50% compared with punctures without a glove. Although double gloving may be beneficial in some cases, in these specific conditions involving deep punctures with hollow-bore needles there is no further decrease obtained by using two glove layers or the equivalent thicker layer ‘blank’ glove.10 These results show that the ‘wiping effect’ of the needle external surface by the glove material described in previous studies is already maximal with a single glove layer.3 The remaining contaminated liquid present inside the hollow portion of the needle is unlikely to be removed by pure mechanical effect, so the volume of blood passing through the glove is directly linked to the inner volume of the hollow-bore needle.

This is consistent with our observation of a linear relationship between volume of blood transmitted and puncture depth, and the relationship between increased transferred volume and the square of the inner needle diameter. Indeed, assuming the inner volume is that of a cylinder, its variation is linear with its height and with its base surface.

An important point to notice is that the ‘wiping effect’ of the tool is a mechanical constriction process influenced by material viscoelastic characteristics, failure mechanism and geometry of the puncturing item. If suture needles can be partly cleansed by a single or, even better, a double glove, other sharp objects (scalpel blades, bone fragments, etc.) with more complex geometries are not subject to the same mechanical cleaning process because of hidden, sometimes porous, less accessible surfaces. As a result, the use of a hollow-bore needle could be considered as a relevant model (worst case scenario) to reproducibly simulate an injury caused by such objects.

Applying our model, the protection afforded by the G-VIR® glove was assessed using HSV1 as a surrogate for enveloped viruses at a concentration relevant to most blood titres likely to be faced in clinical activity.11,12 In agreement with previous results, we confirmed that the G-VIR® glove significantly decreases the transmitted viral load, from 74 to 92% depending on the needle bore, compared with standard gloves of the same thickness.3 Furthermore, these results were obtained using blood, thus taking into account interfering phenomena between proteins and quaternary ammonium salts known to reduce the activity of the disinfectant.13 As the volume of the collecting medium in the microtube led to a massive and rapid dilution of the disinfectant, effectively rapidly quenching its activity, the biological effect measured is a result of the brief contact of the virus with the disinfecting agent in the needle. This rapid activity is consistent with previous in-vivo results.5 This efficiency is not affected by temperature in the range (22–37 °C).

The efficacy is maintained even if the glove is damaged by large needles. This is in fact a direct consequence of the mechanism of action, by which the amount of disinfecting agent expelled increases with the size of the puncturing item.4

We have previously reported that the G-VIR® glove is equally effective against HSV1, feline immunodeficiency (FIV) and bovine viral diarrhoea (BVDV) viruses when they are used as surrogates for HIV and HCV, respectively, under these specific conditions, both in vitro and in vivo.5 It is now commonly admitted that, considering the small volumes involved during a needlestick accident, a significant reduction in the number of viruses transmitted also results in a significant decrease of the contamination risk. In these circumstances, the G-VIR® glove currently offers the highest standard of protection for healthcare workers exposed to sharp injuries with blood from enveloped virus-infected patients.

Implementation of this new glove could be considered as a result of a global risk assessment approach, taking into account not only blood exposure accident frequencies and gravities, but also availability of adapted safety medical devices and cost issues in order to define specific risk-related situations.
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Appendix A: Supplementary data

A supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhin.2007.05.008.

Conflict of interest statement

R. Krikorian, A. Lozach-Perlant, P. Hoerner and P. Sonntag are employees Hutchinson SA, producing and selling the G-VIR® glove in Europe.

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