

# Randomized Trial Comparing Vaccinia on the External Surfaces of 3 Conventional Bandages Applied to Smallpox Vaccination Sites in Primary Vaccinees

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**Background.** Concern about accidental contact transmission after smallpox vaccination has prompted various recommendations regarding vaccination site coverage.

**Methods.** On days 6–8 after their first-ever smallpox vaccination, 63 adult subjects were randomized to apply a self-adhesive bandage ( $n = 20$ ), gauze with adhesive tape ( $n = 21$ ), or gauze with a semipermeable dressing ( $n = 22$ ) over the vaccination site for a mean of  $8 \pm 2$  h. Swabs from the external bandage surfaces and the vaccination sites were then assessed by real time vaccinia-specific polymerase chain reaction (PCR) in blinded fashion.

**Results.** Among 58 subjects completing the study, PCR results were positive for the vaccination site in 55 (94.8%) and on 10 swabs (17.2%) from external bandage surfaces. There were no differences among the 3 bandages ( $P = .57$ ).

**Conclusions.** At 7 days after smallpox vaccination, a peak time for vaccinia shedding, a self-adhesive bandage was as effective as 2 bulkier, less convenient bandages in limiting PCR-detectable virus on the external surface.

Recent concerns about biological terrorism have prompted the United States to institute a smallpox immunization program, starting with soldiers, health care workers, and first responders. Given that >115 million Americans have never been immunized with vaccinia [1], a primary concern among this first wave of recipients, many of whom are also vaccinia naive, is accidental virus transfer during postvaccination shedding to naive family members, household contacts, or patients, especially those at higher risk for serious reactions, such as atopic or immunosuppressed persons [2–4]. Vaccinia-naive individuals shed vaccinia for a longer

duration and at higher titers than do vaccinia-primed individuals, potentially increasing the risk for accidental viral transfer [5–7]. Since the beginning of the armed forces smallpox vaccination program in December 2002, there have been 30 reported cases of accidental contact transmission [7, 8].

Recommendations for covering the vaccination site vary. The World Health Organization (WHO) does not advocate bandaging [9]. The US Centers for Disease Control and Prevention (CDC) recommend a porous gauze dressing in non-health care settings or a gauze dressing covered with a semipermeable membrane for health care workers [10]. It is unclear whether the type of bandage applied to a vaccination site affects viral passage to the external surface. In this study, 7 days after vaccination of vaccinia-naive soldiers, we applied 1 of 3 conventional bandage types to vaccination sites. After a period of daily routine activity, we obtained swabs from the external bandage surfaces and the vaccination site reactions for analysis by real-time vaccinia-specific PCR and culture.

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## PATIENTS AND METHODS

**Study participants.** The study was a randomized, semi-blinded trial conducted from October 2003 through January 2004 at Dwight D. Eisenhower Army Medical Center (Fort Gordon, GA). The hospital institutional review board approved the study, and all participants provided informed written consent.

Eligible participants were healthy, vaccinia-naive, active-duty men and women aged 18–35 years who received smallpox immunization during predeployment screening. We considered participants to be vaccinia naive if they had no evidence of smallpox vaccine documentation in the medical record, were born after 1970, had no Jennarian scar, and began military active duty service after 1984. Immunizations were administered by a standard scarification manner with a vaccinia-coated bifurcated needle pressed 3 times into the skin of the upper arm (Dryvax; Wyeth Laboratories; lot numbers 4002071 and 4002074) [11]. On the day of bandage randomization, all vaccination sites were assessed by one experienced investigator for a major reaction or “take,” a desirable sign indicative of vaccine success defined by the WHO as a pustule, eschar, or ulcer surrounded by an area of inflammation or congestion [10]. Subjects lacking a major reaction were excluded from further participation.

**Procedures.** Six, 7, or 8 days after vaccination, participants with a major reaction were randomly assigned to receive 1 of 3 bandages, applied by a single investigator. The areas of the major reaction, including the pustule and surrounding erythema, were calculated by the mean of the orthogonal diameters. Group 1 received a  $1.9 \times 7.6$ -cm self-adhesive bandage (Band-Aid; Johnson & Johnson); group 2 received 1 unfolded  $5.1 \times 5.1$ -cm gauze (Kendall Clarity Gauze Sponge) covered by a  $2.5 \times 12.7$ -cm piece of adherent tape (Transpore surgical tape; 3M); and group 3 received 1 unfolded  $5.1 \times 5.1$ -cm gauze (Kendall Clarity Gauze Sponge) covered by a  $5.1 \times 7.0$ -cm semipermeable membrane (Polyskin II Transparent Dressing; Kendall). After bandage application, participants resumed their daily activities but were asked not to bathe or remove the bandage.

After  $8 \pm 2$  h, a single investigator rolled 1 dry swab (Sterile Rayon Tipped Applicator; Harwood Product) over the outer surface area of the bandage with even pressure, as described elsewhere [5]. The bandage was removed and a second dry swab was rolled over the reaction pustule in similar fashion. Each swab was placed in a sterile vial containing viral transport media (VTM; Viomed Laboratories). Aliquots of VTM-containing swab material were stored at  $2^{\circ}\text{C}$ – $8^{\circ}\text{C}$  for up to 3 weeks until the PCR was done, and the remaining VTM was stored at  $-70^{\circ}\text{C}$  for up to 1 month until culture was done.

Participants used a Likert scale ranging from 0 (none) to 5 (severe) to describe the degree of bandage discomfort, and they

rated pain (muscle, joint, and chest), headache, and vaccination site pruritis on a 0 to 10 scale (none to most severe).

**PCR and viral cultures.** A single investigator blinded to sample origin conducted all molecular analyses and cultures on the swab samples in a Level 2 Biosafety Laboratory. Real-time Smart Cycler System PCR (Cepheid) was conducted according to the institution’s laboratory response network (LRN) protocol by means of reagents and procedures similar to those developed at the CDC (Richard F. Meyer, Inger Damon, and Yu Li). The LRN protocol was developed as part of an algorithm to rule out biological terrorist activity and to assess smallpox vaccine-related adverse reactions. We conducted DNA extractions (QIAmp DNA Mini Kit; Qiagen) and fluorogenic assays in similar fashion to those described elsewhere [12]. The vaccinia primer sequence is proprietary information. A fast thermal cycling program was provided, and fluorescence was measured at the end of each annealing step. Samples were considered positive for vaccinia if the fluorescence curve crossed a predetermined fluorescence line within the allotted cycle time.

After PCR analysis, all bandage surface samples that had positive PCR results were matched with randomly selected same-group PCR-negative surface swabs and cultured in A549 human lung carcinoma cells (Viomed Laboratories). Five randomly selected PCR-positive major reaction swabs and a Dryvax-positive control containing  $\sim 5.0 \log_{10}$  pock-forming units (pfu)/mL were also cultured. Cultures were kept at  $35^{\circ}\text{C}$  and observed daily by an experienced technician blinded to sample origin for cytopathic effect (CPE) for up to 14 days, graded from 0% to 100% [13].

We quantified  $\log_{10}$  pfu/mL for swabs by performing a logarithmic regression analysis comparing real-time PCR threshold cycle ( $C_T$ ) values from serially diluted duplicate  $1\text{-}\mu\text{L}$  aliquots of Dryvax (lot 4002074) containing  $\sim 1 \times 10^8$  pfu/mL. A best-fit line ( $R^2 = 0.977$ ) with a range of  $0.3$ – $3.3 \log_{10}$  pfu/mL was established.

**Statistical analysis.** On the basis of 2 studies describing 3 bandage types in a limited number of vaccinated subjects [14, 15], we hypothesized that the proportion of bandages with PCR-detectable vaccinia in groups 1, 2, and 3 would be 30%, 10%, and 5%, respectively. To limit the risk of a type I error at  $\alpha = 0.05$ , estimated target sample sizes were 30–40 subjects per study arm, assigned in a 1:1:1 ratio. Because of uncertainty in the hypothesis proportions, a per-protocol interim analysis was conducted after 60 enrollments to reassess the hypothesis. Further enrollment to reach the target sample size was prevented by a markedly reduced tempo of unit activation at the study site. For “takes,” we estimated that  $>95\%$  would have positive PCR results.

The primary end point, PCR detection of vaccinia DNA on the external bandage surface, was expressed as nominal data

and analyzed by Pearson's  $\chi^2$  analysis. Culture outcomes were expressed as proportions. Vaccine site measurements and patient satisfaction for each bandage were compared with analysis of variance, and scaled symptoms scores were analyzed by the Kruskal-Wallis test. *P* values of  $\leq .05$  were considered significant.

## RESULTS

Subjects completing the study per protocol were similar in age (mean age, 24.7 years), sex (67% were men), race (69% were white), number of days since vaccination (mean, 6.8), and size of the major reaction among the 3 randomized groups. One subject failed to develop a major reaction and was excluded before bandage randomization, and 5 subjects failed to return after bandage application (1 in group 1, 2 in group 2, and 2 in group 3). One subject from group 1 received the bandage 9 days after vaccination and was included in the analysis. All bandages remained completely attached to the vaccination sites during the test period.

Table 1 shows clinical, laboratory, and subjective outcomes. Bandage application times were similar for each group, but group 3 had significantly less bandage discomfort (*P* = .01). Other symptom scores, including muscle pain (*P* = .26), joint pain (*P* = .38), chest pain (*P* = .99), localized pruritis (*P* = .47), and headache (*P* = .16), were not significantly different among the groups. Ten (17.2%) of 58 bandage swabs yielded positive PCR results, with the fewest being in group 3 (*P* = .57). Most major reaction swabs were PCR positive (55 [95%] of 58). The 3 PCR-negative major reaction swabs also had PCR-negative bandage surface swabs.

The group 1 subject who applied the bandage 9 days after vaccination had a positive bandage PCR and was the only participant who had more vaccinia DNA on the bandage surface than the major reaction (1.1 log<sub>10</sub> pfu/mL vs. 0.9 log<sub>10</sub> pfu/mL, respectively). For other PCR-positive bandage samples, the amount of vaccinia (pfu/mL) was 40–60-fold less on the bandage swabs in comparison with the corresponding major reaction swabs, regardless of bandage type (*P* = .86).

Two of 10 PCR-positive bandage samples (one from group 1 and the other from group 2) developed CPE (table 1). Both samples showed CPE by day 7, reaching 100% and 75% of cells, respectively, by day 14. These 2 samples had the highest (1.08 log<sub>10</sub> pfu/mL) and lowest (−0.84 log<sub>10</sub> pfu/mL) PCR-derived virus amounts, respectively, among the 10 PCR-positive bandage swabs. All 5 randomly selected positive vaccinia pustule swabs and the Dryvax control sample developed CPE by 48 h, progressing to 100% by day 5, whereas all randomly selected, group-matched negative bandage surface swabs showed no CPE by day 14.

## DISCUSSION

Our findings suggest that a simple adhesive bandage may be as effective as other bulkier, less convenient gauze-tape or gauze-semipermeable dressings in limiting viral passage from a smallpox vaccination "take" to the external bandage surface over a period of routine activity. This finding was notable because our measurements were obtained 6–8 days after vaccination, coinciding with the middle of the peak period for post-vaccination vaccinia shedding and an optimal time for assessing a "take" [7, 16, 17]. Moreover, our subjects were vaccinia naive,

**Table 1. Clinical, subjective, and laboratory outcomes of 58 subjects who received smallpox vaccinations.**

Variable, outcome measured	All subjects ( <i>n</i> = 58)	Bandage group			<i>P</i> <sup>a</sup>
		1: Band-Aid ( <i>n</i> = 19)	2: Gauze with tape ( <i>n</i> = 19)	3: Gauze with Polyskin II ( <i>n</i> = 20)	
Bandage, mean ± SD					
Time worn, h	7.9 ± 1.0	8.0 ± 1.0	8.0 ± 1.1	7.8 ± 1.0	.88
Bothersome score	1.4 ± 0.8	1.6 ± 0.8	1.6 ± 1.0	1.0 ± 0.0	.01
Bandage surface <sup>b</sup>					
Positive PCR result, no. (%) of subjects	10 (17.2)	4 (21.1)	4 (21.1)	2 (10.0)	.57
Viral titer, mean log <sub>10</sub> pfu/mL	0.3	0.6	0.09	−0.3	.66
No. of cultures showing CPE/no. performed	2/10	1/4	1/4	0/2	.73
Vaccination site "takes"					
Positive PCR result, no. (%) of subjects	55 (95)	19 (100)	17 (89)	19 (95)	.34
Viral titer, mean log <sub>10</sub> pfu/mL <sup>c</sup>	1.7	1.9	1.6	1.5	.90
No. of cultures showing CPE/no. performed	5/5	2/2	1/1	2/2	1

**NOTE.** Band-Aids were manufactured by Johnson & Johnson; Polyskin II is manufactured by Kendall. CPE, cytopathic effect; pfu, pock-forming units.

<sup>a</sup> Comparison of groups 1–3.

<sup>b</sup> PCR-positive bandage swabs (*n* = 10).

<sup>c</sup> For PCR-positive "take" swabs (*n* = 55).

a group known to shed larger amounts of virus than vaccinia-primed persons [5–7]. Among the 10 bandage surfaces with PCR-detectable vaccinia, only 2 were culture positive, suggesting virus particles that reach the external bandage surface may have limited viability and, perhaps, limited potential for accidental transmission. Nonetheless, swabbing technique, sample processing, freeze-thawing, host cell factors, and culture conditions may have affected culture outcomes.

The CDC recommendations for bandaging vaccination sites are largely modeled after 2 descriptive studies [14, 15], but we are unaware of any prospective comparative studies assessing these options. In 1992, Graham et al. [14] found that 18% of single semipermeable membranes covering the “take” site were culture positive, a figure reduced to 3% when gauze was covered by 2 semipermeable membranes. Cooney et al. [15], in a group of 4 patients, described a 0% surface vaccinia culture rate after application of a 1 × 1-cm gauze covered by a 5 × 8-cm semipermeable membrane. More recently, Talbot et al. [18] reported a low incidence (0.65%) of vaccinia on the bandage surface, but the vaccination sites were covered with 2 waterproof occlusive dressings, a relatively inconvenient option. Here, a self-adhesive bandage effectively limited PCR- and culture-detectable virus on the external bandage surface after a period of routine activity, suggesting a simple but reasonably effective option for routine vaccine-site care. The use of a self-adhesive bandage also aligns with recommendations to avoid more occlusive bandages that may cause maceration or bacterial infection [16]. Nonetheless, and in general agreement with our hypothesis, the bulky gauze-semipermeable bandages (group 3) had the smallest proportion of detectable surface virus.

This is the first study to use real-time PCR to detect vaccinia on the bandage surface. The technique performed well, with reproducible positive and negative control sample outcomes, as well as a positive detection rate of ~95% for swabs obtained from “takes,” a rate generally consistent with current orthopoxvirus PCR strategies [12]. Real-time PCR allowed construction of a logarithmic plot to estimate the amount of vaccinia DNA for each swab on the basis of PCR cycle time, a variable that may be useful in some studies.

We measured viral DNA once on bandage surfaces after ~8 h. In practice, however, recipients might be instructed to change their dressing every 1–3 days for up to 3 weeks, coinciding with reduced bandage adhesion or visible exudates. Three weeks of dressing changes complicate compliance, especially if a less convenient, bulkier dressing is used. This underscores the notion that a simple plaster adhesive containing absorbent material might increase compliance, and perhaps reduce the risk of accidental transmission. Strategies to better define the efficacy of these and other bandages for limiting viral passage to

the external bandage surface include application for several days, normal bathing habits, and multiple samplings.

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