원 저

A Quantitative Comparison of Vaccinia Virus Shedding from Conventional Dressing Sites and Vaccination Lesions after Smallpox Vaccination

Sung-Han Kim, M.D.[1], Sang-Gu Yeo, D.V.M.[2], Jae-Hyun Cho, M.D.[1], Ji-Whan Bang, M.D.[1]
Hong-Bin Kim, M.D.[1], Nam-Joong Kim, M.D.[1], Youngmee Jee, M.D., Ph.D.[2]
Haewol Cho, Ph.D.[2], Myoung-don Oh, M.D.[1] and Kang-Won Choe, M.D.[1]
[1]Department of Internal Medicine, Seoul National University College of Medicine
[2]Division of Enteric and Hepatitis Viruses, Center for Infectious Diseases, National Institute of Health, Seoul, Korea

두창백신 접종 후 접종 부위와 드레싱 부위에서 백시니아바이러스 배출량의 정량적인 비교분석

서울대학교 의과대학 내과학교실[1], 국립보건연구원 간염폴리오바이러스팀[2]
김성한[1] · 여상구[2] · 조재현[1] · 방지환[1] · 김홍빈[1] · 김남중[1] · 지영미[2] · 조해월[2] · 오명돈[1] · 최강원[1]

Background : We compared vaccinia virus shedding from the vaccine inoculation site (vaccination lesion) and two sites of a dressing covering the vaccination site; the outer surface of the semipermeable dressing (outer surface) and the inner surface of the semipermeable dressing, that is, the surface of a folded gauze under the semipermeable membrane (gauze surface)
Material and Methods : Subjects were recruited from the volunteers who participated in a clinical trial of the efficacy of a 1:10 dilution of Lancy-Vaxina® (Berna Biotech, Switzerland), and were seen every 2-3 days (days 6, 8, 10, 13, and 15 after smallpox vaccination) for scheduled dressing changes. Swab specimens were obtained from the vaccination lesion, the outer surface, and the gauze surface. Quantitative viral culture assays for these specimens were done.
Results : Vaccinia virus was recovered from 126 (81%) of the 156 vaccination lesion samples collected from the 40 participants. A high virus titer was recovered from the vaccination lesion (geometric mean titer (log_{10})=3.91 on day 8). Of the 39 swab samples obtained from the gauze surface of the gauze, 16 (41%) were positive for virus. An intermediate titer was recovered from the gauze surface (geometric mean titer (log_{10})=0.91 on day 8). Of the 133 swab samples obtained from the outer surface, only one (0.8%) was positive for vaccinia. No virus was recovered from the outer surface on day 8.
Conclusion : Our findings suggest that the addition of a semipermeable dressing to the folded gauze further reduces viral shedding and therefore increases protection.

Key Words : Smallpox, Dressing, Vaccination

INTRODUCTION

Vaccinia can be transmitted from a unhealed vaccination site to other persons by close contact, and can lead to inadvertent inoculation of the vaccine (1). To prevent such transmission, the Centers for Disease

Submitted 2 June, 2006, accepted 21 February, 2007
This study was supported by a grant of the Korea Center for Disease Control and Prevention, Seoul, Republic of Korea (347-6112-162-000-206)
Correspondence : Myoung-don Oh, M.D
28 Youngun-dong, Chongro-gu, Seoul, Korea
Department of Internal Medicine, Seoul National University Hospital
Tel : 82-2-2072-2945, Fax: 82-2-762-9662
E-mail : mdohmd@snu.ac.kr

Control and Prevention (CDC) has suggested the use by health care workers of semipermeable dressings with folded gauze, and also covering vaccination lesions with a folded gauze in other settings (1). However, these recommendations were based on studies that did not address comprehensively whether the vaccinia virus was actually confined by these dressing strategies (2, 3). A few recent studies have considered this issue, but with conflicting results (4-6). We have conducted a prospective observational study to compare the quantities of virus shed from the outer surface of a semipermeable dressing, its inner surface (the surface of folded gauze under the semipermeable membrane), and the vaccination site.

MATERIALS AND METHODS

Subjects were recruited from volunteers who enrolled at Seoul National University Hospital between June and October 2004, and participated in a single-blind, randomized clinical trial[7] for the efficacy of a 1:10 dilution of Lancy-Vaxina® (Berna Biotech, Switzerland). The vaccine was derived from the Lister strain, and the titer of vaccinia virus was $10^{7.7}$ plaque forming units (pfu)/mL. A semipermeable adhesive membrane (Tegaderm®, 3M Korea, Republic of Korea) was applied to all the vaccination sites, and folded gauze was placed underneath the semipermeable dressing when subjects had visible exudates (all but two participants had visible exudates at the vaccination site between day 7 and day 9 after vaccination). However, a semipermeable dressing without folded gauze was applied to the vaccination site for the first 5 days after vaccination and after scab formation because there were no visible exudates in these periods.

All volunteers were seen every 2-3 days (days 6, 8, 9, 13, and 15 after smallpox vaccination) for scheduled dressing changes, but we showed the volunteers how to remove old dressings in case an unscheduled dressing change was needed before the next clinic visit. Because it takes about 2 weeks to form a scab after smallpox vaccination, we monitored viral shedding up to 15 days after vaccination. Specimens for viral culture were obtained at each scheduled visit. For swabbing, we moistened cotton-tipped swabs with transport medium (DMEM with 1% FBS and 1% penicillin & streptomycin), and swabbed about 10 times, 5 times in the horizontal direction and 5 in the vertical direction over the site to be sampled (Figure 1). The investigators (MD Oh or SH Kim) rolled the swabs over the outer surface of the semipermeable dressing before removing the semipermeable membrane (outer surface of semipermeable dressing), and obtained swab samples in this way until a scar had formed. We also carefully lifted the semipermeable membrane off the folded gauze and, obtained a swab sample from the outer surface of the gauze (gauze surface). However, when the folded gauze dressing was so firmly attached to the semipermeable membrane that it could not be removed, we did not obtain this sample. In addition, swabs were rubbed over the bare lesions at the vaccination sites (the vaccination lesion). The specimens were inoculated directly into viral transport medium and frozen at -70℃ for batch processing.

Quantitative viral culture assays were done as described previously (2, 5). All specimens were inoculated directly onto plates coated with monolayered BSC-40 cells at duplicate serial dilutions of 1:1, 1:10, 1:100, and 1:1000. After adsorption for 1 hour at 37℃, each monolayer was overlaid with 1 mL of methyl cellulose in DMEM with 1% fetal bovine serum. After incubating for 2 days at 37℃ in a humidified atmosphere of 5%

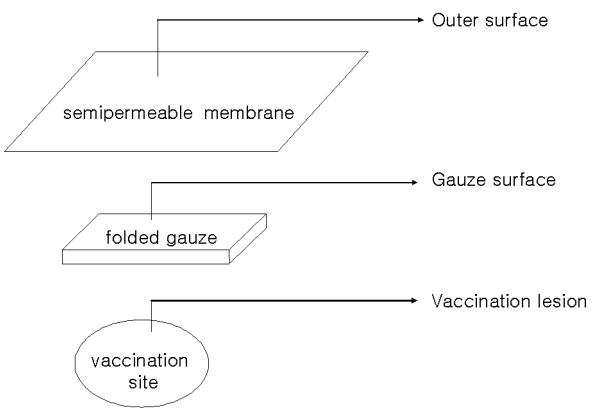

Figure 1. Schematic diagram of the sites of the swabs obtained from the vaccine inoculation site (vaccination lesion), the outer surface of the semipermeable dressing (outer surface) and the inner surface of the semipermeable dressing (gauze surface).

carbon dioxide atmosphere, plates were fixed with 10% buffered formalin phosphate and stained with 2% crystal violet to visualize the presence of vaccinia plaques. Quantitative viral titers were expressed as the average of duplicate counts in $\log_{10}$ plaque-forming units (pfu) per milliliter.

RESULTS

Forty volunteers participated in this study. Mean age (±SD) was 31.2±6.8 years. Of the 40 subjects, 26 (65 %) had been remotely vaccinated, and 14 (35%) were vaccinia-naïve. Fifteen (38%) persons received undiluted smallpox vaccine, and 25 (62%), vaccine diluted 1:10. All vaccinations induced a primary reaction and follow-up was continued until scab formation (an average of 3.7 follow-up visits [95% CI, 3.4-3.9]). Dressings were changed an average of 4.4 times (95% CI, 3.9-4.9) until scab formation. All but two participants had visible exudates at the vaccination site between days 7 and 9 after vaccination, so that we had to place a folded gauze underneath the semipermeable dressing. Autoinoculation occurred in one volunteer. There were no contact transmissions.

Vaccinia virus was recovered from 126 (81%) of the 156 vaccination lesion samples collected from the 40 participants. All 40 participants were positive for virus on at least one occasion between days 6 and 15 after vaccination. A high virus titer was recovered from the vaccination lesion (geometric mean titer ($\log_{10}$)=3.91 on day 8). Of the 39 swab samples obtained from the surface of the gauze, 16 (41%) were positive for virus. An intermediate titer was recovered from the surface of the gauze (geometric mean titer ($\log_{10}$)=0.91 on day 8). About half of the folded gauze dressings stayed firmly attached to the semipermeable membranes and were not sampled. Of the 133 swab samples obtained from the outer surface, only one (0.8%) was positive for vaccinia. No virus was recovered from the outer surface on day 8. The quantitative results for vaccinia virus on the outer surface, the gauze surface, and the vaccination lesion are shown in Figure 2. Data on the dependence of viral shedding on the vaccine dilution used and previous vaccination status are given in Figure 3.

DISCUSSION

Our data suggest that covering the vaccination site with a gauze and semipermeable dressing is associated with a low rate (0.8%) of recovery of live vaccinia

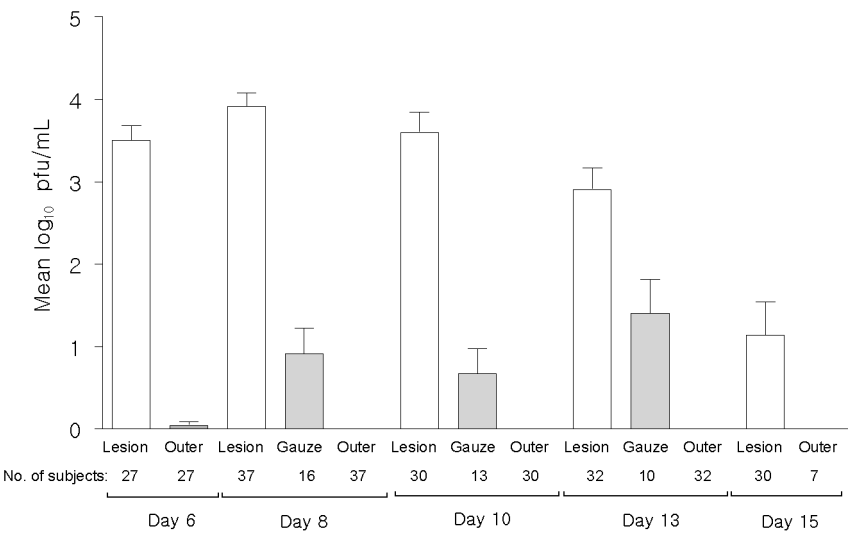

Figure 2. Virus yields from the outer surface, gauze surface, and vaccination lesion. "Lesion" denotes swab samples from the vaccinia inoculation sites, "Outer" those from the outer surface of the semipermeable dressings, and "Gauze" those from the inner surface of the semipermeable dressings. Geometric mean titers ($\log_{10}$) are given with standard errors.

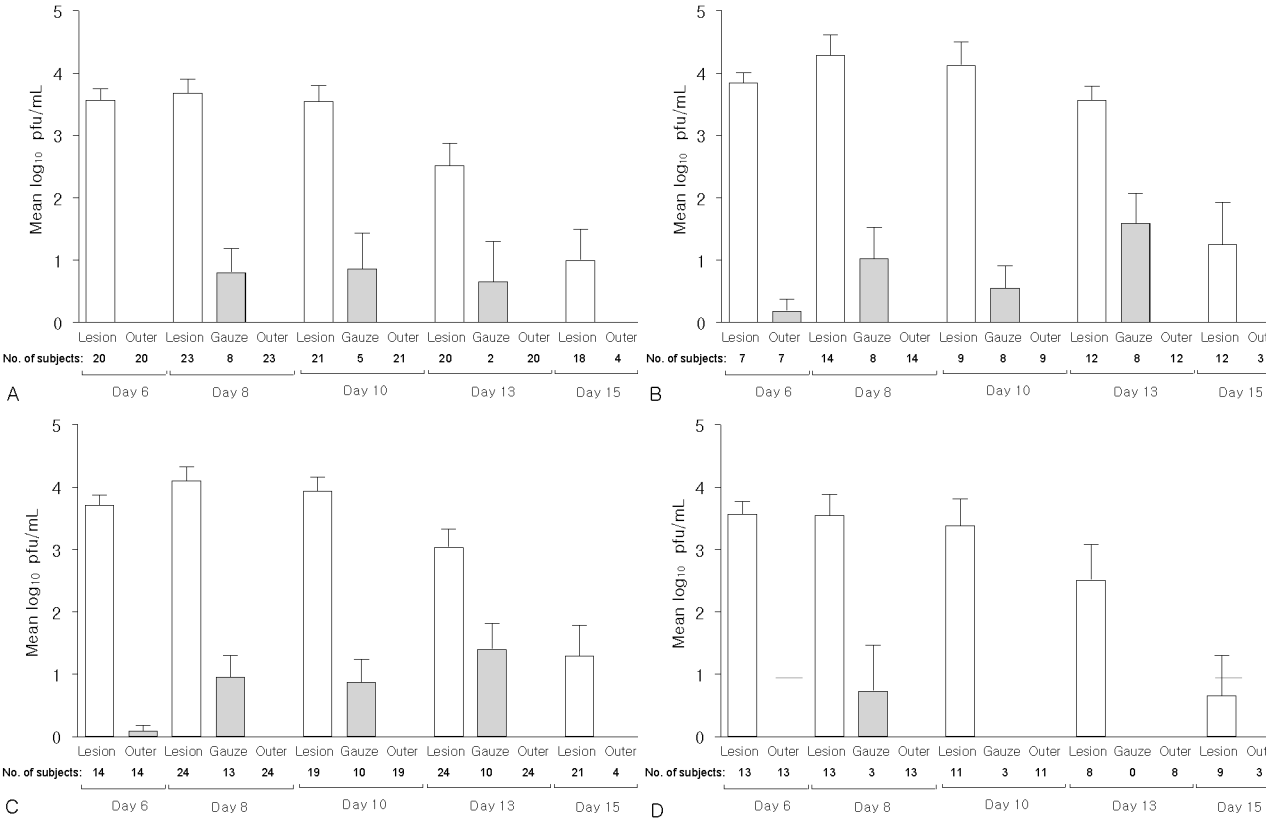

Figure 3. Virus yields from the outer surface, gauze surface, and vaccination lesion as a function of vaccine dilution (A]: undiluted group; B]: 1:10 dilution group) or previous vaccination history (vaccinia-naïve group C] and previously vaccinated group D]). "Lesion" denotes swab samples from the vaccinia inoculation sites, "Outer" those from the outer surface of semipermeable dressings, and "Gauze" those from the inner surface of the semipermeable dressings. Geometric mean titers (log_{10}) are given with standard errors.

virus from the outer surface of the dressing. Only a few other studies have addressed this issue. Cooney et al. reported that all swabs from the outer surfaces of dressings consisting of gauze and a transparent dressing (OpSite®) were negative (190 swabs) after vaccination with a recombinant vaccinia virus expressing HIV envelop glycoprotein (2). In a similar HIV vaccine trial, Graham et al. found that 12 (18.2%) of 66 swabs of the outside of dressings consisting of a transparent membrane (OpSite®) only, were positive for vaccinia virus, and that the addition of a second occlusive dressing and sterile gauze reduced the rate of positive dressing cultures to 3.0% (3 of 103 swabs) (3). However, these two studies did not address comprehensively the quantity of viral shedding from dressing sites. Recently, Talbot et al. reported that 6 (0.65%) of 918 samples taken from the outermost surface of a pair of occlusive bandages, an initial transparent bandage (OpSite®) and an outer semipermeable bandage (Tegarderm®), were positive for vaccinia virus (5). However, the dressing strategy (two semipermeable bandages) in that study (5) was different from that used in our study and was not one recommended by the CDC. Although Hepburn et al. (4) recently compared a gauze and semipermeable dressing (Tegarderm®) with a non-occlusive dressing, they reported a rather high positive culture rate (7% [10/135 swabs]) for Tegarderm® with a double layer of gauze, and therefore recommended that appropriate precautions be taken if these dressings are used. Waibel et al. (6) also reported high positive PCR rate (10% [2/20 swabs]) for a semipermeable dressing over folded gauze. To our knowledge, ours is the first report which comprehensively evaluates the quantities of viral shedding from the conventional dressing strategy recommended by the CDC and demonstrates an extremely low rate of vaccinia recovery from the occlusive dres-

sings (Table 1). Moreover we have provided a more detailed kinetic analysis than most of the earlier studies. This should provide better insight into the recovery of virus from dressings used to cover vaccinia inoculation sites and hence into the rationale for the choice of dressings in various settings.

We were not able to make a direct comparison of the recovery of vaccinia from sites covered by occlusive bandages (i.e. folded gauze plus semipermeable membrane) versus recovery from non-occlusive dressings (i.e. folded gauze only) because we used only one type of vaccination dressing strategy. However, we found that the virus titers from the outer surface of the folded gauze were substantially lower than those from the vaccination lesions themselves (a reduction of >1,000-fold in titer), and that those from the outer surface of the semipermeable dressing over the folded gauze were lower still (a reduction of >10,000-fold in titer). So we can conclude that the use of a semipermeable dressing with folded gauze for health-care workers is more appropriate to prevent nosocomial transmission of vaccinia virus than a non-occlusive gauze dressing for non health-care persons.

We applied a sterile gauze underneath the semipermeable membrane when exudates became apparent, and this occurred in nearly all subjects 7 to 9 days after vaccination, coincident with the middle of the peak period for post-vaccination virus shedding. A previous study reported a trend towards a lower prevalence of culture positivity on dressings without visible exudates, compared to those with visible exudates (4). However, this comparison may not be reliable if the samples were obtained at different times post-vaccination (sampling times were not specified).

Some authors have found that semi-permeable dressings such as Tegaderm® increase the frequency of adverse local skin reactions (8). In the present study, a few individuals presented with local itching or a burning sensation in the Tegaderm® attachment area. However, none of the 112 volunteers in the Lancy-vaxina® dilution trial (7) required an alternative dressing because of significant local skin irritation. In addition, Regules et al. have reported that the use of a semipermeable dressing seems to prolong the time to scab separation and possibly the duration of infectivity (9). In general, the scab is not believed to be highly infectious, although it contains some virus (1). Further comparisons of occlusive and non-occlusive dressings with respect to viral shedding are required to settle this issue.

Interestingly, in the present study one person developed an autoinoculation lesion in the upper back, and another lesion in the mid-axillary line to the side of the vaccination site. The reason for the autoinoculation despite the fact that there was no detectable virus on the outside of his dressing and the usual amount of shedding from his vaccination site ($10^{3.2}$ pfu/mL, $10^{4.7}$ pfu/mL, and $10^{3.2}$ pfu/mL on days 8, 10, and 13, respectively) is unclear. However, autoinoculation not only

Table 1. Literature Review of Viral Shedding for Dressings Applied to Smallpox Vaccination Sites

Author, year, publication [Reference]	Trial profile	Dressing strategy	Quantitative culture	Main findings-vaccinia positive culture rate
Cooney EL, et al. 1991 *Lancet* [2]	HIV vaccine trial	OP Site® with gauze apply*	No	0% (0/190 swabs)
Graham BS, et al. 1992 *J Infect Dis* [3]	HIV vaccine trial	Op Site® only an addition of a second permeable membrane plus gauze*	No	18% (12/66 swabs)→ 3% (3/103 swabs)
Hepburn MJ, et al. 2004 *Am J Infect Control* [4]	Dryvax trial	Tegaderm® with gauze* vs. gauze only[a]	No	7% (10/135 swabs) vs. 23% (15/64 swabs)
Talbot TR, et al. 2004 *Clin Infect Dis* [5]	Dryvax trial	Double semipermeable dressing	Yes	0.65% (8/916 swabs)
Waibel KH, et al. 2004 *Clin Infect Dis* [6]	Dryvax trial	Band-Aid® vs. Gauze with tape* vs. Gauze with Polyskin II®*	Yes	21% (4/19 swabs)† vs. 21% (4/19 swabs)† vs. 10% (2/20 swabs)†
Current study	Lancy-Vaxina trial	Tegaderm® with gauze apply*	Yes	0.8% (1/133 swabs)

*The dressing strategy recommended by CDC
† Positive PCR result

requires transmissible live vaccinia virus but also direct inoculation of the virus into the mucosa or damaged skin. Hence, numerous unmeasured factors may affect its occurrence.

One might anticipate that the vaccine dilution used or previous vaccination status could affect the extent of viral shedding from vaccination or dressing sites. As shown in our previous report (7), we found that no significant differences in viral shedding between the 1:1 diluted and 1:10 dilution vaccination groups, and that the lesions of the vaccinia-naïve group showed a trend toward greater viral shedding than the remotely-vaccinated group. However, these subgroup analyses also support the conclusion that a folded gauze dressing decreases viral shedding by two to three logs and the addition of a semipermeable membrane reduces shedding further.

Our study has a number of limitations. Subjects received a different vaccinia strain (Lister) from the vaccine strain (New York City Board of Health) used in most previous studies (2-6, 8, 9). Thus, viral shedding by these 2 different strains is not directly comparable. Moreover, our data on the Tegaderm® dressing are not necessarily applicable to other semipermeable dressings. However, we found that a folded gauze dressing decreases viral shedding by two to three logs and that the addition of a semipermeable membrane further reduces shedding.

ABSTRACT

목 적 : 두창백신 접종 후 일반적으로 접종 부위에 거즈를 먼저 덮고, 그 위에 반투과막을 덮어주는 드레싱을 권장하고 있다. 저자들은 이러한 드레싱 방법의 효율성을 알아보기 위해서 두창백신 접종 후 접종 부위, 거즈 바깥부위, 반투과막 바깥부위에서 각각 분비되는 바이러스양을 정량적으로 비교하였다.

재료 및 방법 : Lancy-Vaxina® (Berna Biotech, Swiss) 두창백신 1:10 희석연구에 참여한 자원자들 중에서 접종부위에 정해진 기간에 검체 채취에 동의한 40명을 대상으로 연구를 진행하였다. 참가자들은 두창백신 접종 후 6, 8, 10, 13, 15일에 각각 방문하여 드레싱을 교체하였다. 드레싱을 교체하면서 두창백신 접종 부위, 거즈 바깥부위, 반투과막 바깥부위에 면봉을 이용해서 각각 검체를 채취하였다. 검체는 냉동 보관한 후 plaque assay를 통하여 정량적으로 바이러스 역가를 측정하였다.

결 과 : 총 40명의 자원자가 본 연구에 참가하였다. 백신 접종 부위에서 채취한 156 검체 중 126건(81%)에서 백시니아바이러스가 검출되었다. 백신 접종 부위에서 접종 8일째 바이러스 역가의 평균(geometric mean titer (log_{10}))은 3.91 pfu/mL이었다. 거즈 바깥부위에서 총 39 검체가 얻어졌고, 이 중 16건(41%)에서 백시니아바이러스가 검출되었다. 거즈 바깥부위에서 접종 8일째 바이러스 역가의 평균은 0.91 pfu/mL이었다. 반투과막 바깥부위에서 총 133 검체가 얻어졌고, 이 중 1건(0.8%)에서 백시니아바이러스가 검출되었다. 접종 8일째 거즈 바깥부위에서는 바이러스가 검출되지 않았다.

결 론 : 두창백신 접종 후에 거즈를 덮고 그 위에 반투과막을 덮어주는 드레싱 방법은 백시니아바이러스 배출을 효과적으로 줄여줄 수 있음을 확인하였다.

ACKNOWLEGEMENTS

We thank the volunteers of this study. We also thank Chong-Hee Kim and Hyun-Woo Han for the recruitment of the volunteers and the administrative support, and Dr. Seon-Hee Lee for invaluable help.

REFERENCES

1) Cono J, Casey CG, Bell DM : *Smallpox vaccination and adverse reactions: Guideline for clinicians. Morb Mortal Wkly Rep 52:1-28, 2003*
2) Cooney EL, Collier AC, Greenberg PD, Coombs RW, Zarling J, Arditti DE, Hoffman MC, Hu SL, Corey L : *Safety of and immunologic response to a recombinant vaccinia virus vaccine expressing HIV envelope glycoprotein. Lancet 337:567-72, 1991*
3) Graham BS, Belshe RB, Clements ML, Dolin R, Corey L, Wright PF, Gorse GJ, Midthun K, Keefer MC, Roberts NJ : *Vaccination of vaccinia-naive adults with human immunodeficiency virus type I gp 160 recombinant vaccinia virus in a blinded, controlled, randomized clinical trial. J Infect Dis 166:244-52, 1992*
4) Hepburn MJ, Dooley DP, Murray CK, Hospenthal DR, Hill BL, Nauschuetz WN, Davis KA, Crouch HK, McAllister CK : *Frequency of vaccinia virus isolation on semiperameable versus nonocclusive dressings covering smallpox vaccination sites in hospital per-*

sonnel. Am J Infect Control 32:126-30, 2004

5) Talbot TR, Ziel E, Doersam JK, LaFleur B, Tollefson S, Edwards KM : *Risk of vaccinia transfer to the hands of vaccinated persons after smallpox immunization. Clin Infect Dis 38:536-41, 2003*

6) Waibel KH, Ager EP, Topolski RL, Walsh DS : *Randomized trial comparing vaccinia on the external surfaces of 3 conventional bandages applied to smallpox vaccination sites in primary vaccinees. Clin Infect Dis 39:1004-7, 2004*

7) Kim SH, Yeo SG, Jang HC, Park WB, Lee CS, Lee KD, Kim HB, Kim NJ, Kim YT, Jee Y, Cho H, Oh MD, Choe KW : *Clinical responses to smallpox vaccine in vaccinia-naive and previously vaccinated populations: undiluted and diluted Lancy-Vaxina vaccine in a single-blind, randomized, prospective trial. J Infect Dis 192:1066-70, 2005*

8) Crittenton E, Davis A, Hoffmann KK, Rutala WA, Weber DJ : *Semipermeable dressing used to cover smallpox vaccination sites as a cause of skin damage. Infect Control Hosp Epidemiol 25:96, 2004*

9) Regules JA, Dooley DP, Hepburn MJ, Van De Car DA, Davis KA, McAllister KC, Hospenthal DR, Murray CK, Fofaria R, Ekstrand JR, Crouch HK : *The effect of semipermeable dressings on smallpox vaccine site evolution. Am J Infect Control 32:333-6, 2004*