

litis. Patients who survive begin to defervesce 2-3 weeks after onset of the disease. Temporary or permanent unilateral or bilateral deafness that occurs in a third of Lassa patients during convalescence is not associated with the severity of the acute disease (Cummins et al., 1990; Rybak, 1990).

[0009] Potential for use of arenaviruses as bioweapons. In addition to high case fatality rates, arenaviruses have many features that enhance their potential as bioweapons. Arenaviruses have relatively stable virions, do not require passage via insect vectors, are spread easily by human-to-human contact and may be capable of aerosol spread or other simple means of dispersal. The high prevalence of Lassa fever in western Africa coupled with the ease of travel to and from this area and endemic areas for MACV, JUNV, GUAU, SABV and other highly pathogenic arenaviruses permits easy access to these viruses for use as a bioweapon. A cluster of hemorrhagic fever cases in the United States caused by any arenavirus would be a major public health incident. Because febrile illnesses are common, the absence of reliable diagnostic tests would greatly increase the impact of the attack and permit wider dissemination via human-to-human contact. The potential use of LASV and other arenaviruses as a biological weapon directed against civilian or military targets necessitates the commercial development of effective diagnostics.

[0010] Treatment/prevention of arenavirus infections. The antiviral drug ribavirin is effective in the treatment of Lassa fever if administered early in the course of illness (Johnson et al., 1987; McCormick et al., 1986). Ribavirin administered to patients with a high virus load (and therefore a high risk for mortality) within the first six days of illness reduced the case-fatality rate from 55% to 5% (McCormick et al., 1986). Several anecdotal reports suggest that this drug can also be effective against other arenaviral hemorrhagic fevers (Barry et al., 1995; Kilgore et al., 1997; Weissenbacher et al., 1986a; Weissenbacher et al., 1986b). The efficacy of prophylactic treatments for Lassa fever is unknown, although it has been suggested that people with high-risk exposures be treated with oral ribavirin. Passive transfer of neutralizing antibodies early after infection may also be an effective treatment for Lassa and other arenaviral hemorrhagic fevers (Enria et al., 1984; Frame et al., 1984; Jahrling, 1983; Jahrling and Peters, 1984; Jahrling, Peters, and Stephen, 1984; Weissenbacher et al., 1986a). The dependence of effective treatment on early diagnosis provides another strong rationale for improving arenavirus diagnostics. No arenavirus vaccine is currently available, although vaccines against LASV and JUNV are in development. Effective diagnostic assays are absolutely essential for development and field testing arenaviral vaccines.

[0011] Diagnostic procedures for arenaviruses. Virus isolation is likely to be the most sensitive assay for detection of LASV and other arenaviruses. However, LASV cannot be uniformly isolated from all acute cases (Bausch et al., 2000; Johnson et al., 1987). Virus culture is too time-consuming for bioterrorism scenarios or clinical settings given the urgency that effective treatment requires. The diversity amongst LASV isolates and the number of other arenaviruses that are potential bioterrorism agents suggests that it may be impractical to develop a useful RT-PCR strategy for rapid detection (Archer and Rico-Hesse, 2002; Bowen, Peters, and Nichol, 1997; Niedrig et al., 2004). The recent International Quality Assurance Study on the Rapid Detection of Viral Agents of Bioterrorism by RT-PCR methods recently found that only a fraction (21-50%) of established biodefense laboratories

could detect common strains of LASV in samples containing fewer than 5000 copies/ml (Niedrig et al., 2004). Furthermore, PCR methods require instrumentation, expertise and facilities generally not available in LASV endemic areas of West Africa (Demby et al., 1994; Lunkenheimer, Hufert, and Schmitz, 1990; Trappier et al., 1993). Our prior results (Bausch et al. 2000) strongly suggest that antigen-capture and IgM-capture ELISA provide the most sensitive and specific serologic tests for acute Lassa virus infection as well as useful prognostic information. We anticipate that similar assays can be developed for New World arenaviruses, which also have high potential for use as bioterrorism agents. This application is based on the premise that, as was the case with advanced generation HIV antibody tests, arenavirus ELISA can be developed with superior sensitivity and specificity compared to currently available noncommercializable assays. Prior studies readily demonstrated the feasibility of this approach (Barber, Clegg, and Lloyd, 1990; Hufert, Ludke, and Schmitz, 1989; Jahrling, Niklasson, and McCormick, 1985; Krasko et al., 1990; Meulen et al., 2004; Vladyko et al., 1990). Additional advantages of ELISA-based diagnostics include their ease of standardization and use (in comparison to PCR-based assays), and their applicability to the diagnosis of numerous other diseases. It should be possible to combine LASV detection with detection for selected pathogens that have a clinical presentation similar to Lassa fever such as Ebola virus or dengue virus. ELISA can be converted to formats that would be especially valuable for rapid diagnosis during an incident of bioterrorism and could be used in technology-poor regions such as West Africa.

[0012] Need for the invention. The scientific literature describing the expression of LASV proteins in prokaryotic systems, such as *E. coli*, report only expression of polypeptide fragments. The expression of truncated forms of LASV nucleocapsid protein in *E. coli* BL21 (DE3) has been reported by Jan ter Meulen et al. (1998 and 2000). In these reports Jan ter Meulen et al. stated that "Neither the whole NP nor the N terminus (amino acids [aa] 1 to 139) could be expressed (data not shown), but a truncated protein (aa 141 to 569) was abundantly overexpressed, extracted from insoluble inclusion bodies with 8 M urea, and purified by nickel-chelate chromatography". The expression of full length LASV nucleocapsid protein in insect cells mediated by infection with a recombinant baculovirus encoding the entire open reading frame of this polypeptide has been reported by L. S. Lukashovich et al. (1993). Furthermore, at the time of this invention no reports in the literature have described expression of truncated fragments or full length LASV GP1 or GP2 polypeptides in prokaryotic systems.

[0013] Thus, development of broad encompassing and highly specific immunological-based diagnostic procedures that use recombinant LASV proteins requires successful expression, purification, and characterization of full length versions of NP, GP1, and GP2. Full length expression of each polypeptide, as outlined in this application, required a rationally designed and empirically optimized approach that utilized specific *E. coli* strains, fusion to a partner protein that both stabilized expression and could be used as a purification domain, expression of rare tRNA codons in the bacterial cell to support efficient translation of the recombinant LASV proteins, and a matrix-based identification of culture medium, induction, and temperature conditions. In addition, localization of recombinant LASV proteins to two independent cellular compartments was investigated. Furthermore,