Statistical analysis

Statistical analyses were performed using GraphPad Prism version 5.00 for Mac OS X (GraphPad Software Inc., La Jolla, CA, USA). Student t-test and One-Way ANOVA with Bonferroni Multiple Comparison post-hoc test was used to determine the statistical significance of differences among mean values. A statistical significance criterion significance level of $p < 0.05$ was used.

Results

Sera of people with no clinical history of loxoscelism recognize *L. laeta* venom

In order to get a general view of immunoreactivity, the serum samples from individuals with loxoscelism (Group 1) and without loxoscelism (Group 2) were evaluated. Initially, the serum pools from Group 1 and Group 2 were used, and the detection of *L. laeta* venom by both groups was evaluated using dot blot. The pool of sera from the Group 1 was able to detect *L. laeta* venom. Meanwhile, the pool of sera from Group 2 was also able to recognize the venom (Fig. 1a). Incubation with PBS and pre-immune mouse serum did not show reactivity, whereas mouse anti-*L. laeta* venom serum showed a marked reaction.

In order to determine whether venom detection by the Group 2 pool of samples was due to the presence of individual serum that could present specific antibodies against *L. laeta* venom, we evaluated the detection of each individual's serum using dot blot. All ten samples from patients with loxoscelism could detect *L. laeta* venom (Fig. 1b). In comparison, among the 30 samples from the group without loxoscelism, 18 of them showed detection levels similar to the sera from the loxoscelism group. In addition, five samples (dots C2, D2, B3, C3, C4, and C5) had higher detection levels than those observed in the loxoscelism group. In contrast, dots F3, A4, G4, H4, and H5 showed lower levels of detection (Fig. 1b; Additional file 1A). Strong detection was observed with mouse anti-*L. laeta* venom immune serum (dot A6) and monoclonal anti-rLlPLD1 (dot B6). Detection was not observed with pre-immune mouse serum (dot C6).

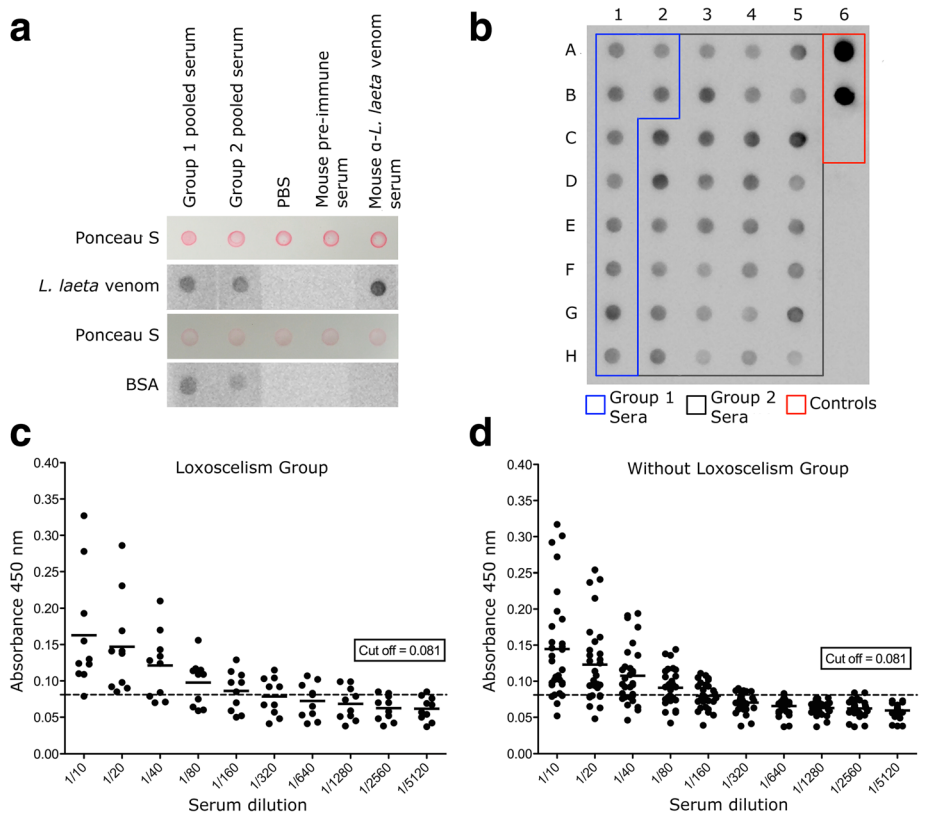

Fig. 1 Detection of *Loxosceles* spider venom by sera from Group 1 and Group 2 by dot blot and ELISA. **a** Dot blot for detection of *L. laeta* venom incubated with serum pools from Group 1 and Group 2 (1:1000 dilution). **b** Representative dot blot of *L. laeta* venom incubated with individual serum from Group 1 (blue line, dots A1-B2) and Group 2 (black line, dots C2-H5); controls (red line): monoclonal antibody 7E4-D2 anti-rLlPLD1 (1:50,000 dilution) (dot A6), polyclonal mouse anti- *L. laeta* venom serum (1:10,000 dilution) (dot B6), pre-immune mouse serum (1:1000 dilution) (dot C6). **c** Indirect ELISA for the titration of Group 1 sera that recognize *L. laeta* venom. **d** Indirect ELISA for titration of sera from Group 2 that recognize *L. laeta* venom