An improved flow cytometry assay with the potential application for serodiagnosis of Chagas' disease

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Chagas disease is caused by the protozoan parasite Trypanosoma cruzi. T. cruzi infection is usually controlled by a highly effective immune response, which does not eliminate the parasite from the vertebrate host, resulting in a low parasitemia and persistent tissue parasitism. In this work we proposed the validation of an improved serodiagnosis methodology based on flow cytometry. This methodology has the potential to be used in the specific diagnosis of T. cruzi infection and could also be optimized to identify co-infections. To standardize this methodology, three recombinant proteins derived from the MASP family (mucin-associated surface protein), which is expressed in trypomastigotes of T. cruzi, were coupled to fluorescent microspheres. To confirm the coupling, anti-MASP sera generated in BALB/c mice were used. The analysis was performed using the MFI calculation and anti-MASP sera showed much higher values compared to the negative control, indicating that the coupling was successful. We have also performed two tests with these MASP recombinant proteins coupled to different fluorescent microspheres and with pools of sera from mice infected with T. cruzi. In the first test, each individually coupled MASP was incubated with the sera. In the other one, all MASPs coupled to beads with different fluorescence, forming a mix, were incubated simultaneously with the sera. The profile of reactivity was very similar in both tests, indicating that both methods are effective and could potentially be used in the serodiagnosis of Chagas disease. In addition, this result also suggests that this methodology could be optimized to identify co-infections by coupling antigens derived from different pathogens in distinct fluorescent beads.