

### **Preparation of experimental dried blood spots**

To assess the diagnostic accuracy of the *g*-iELISA on dried blood spots (DBS), experimental DBS were prepared as follows.

Venous blood was taken on heparin from a trypanolysis-negative O+ donor and divided into 500  $\mu$ l volumes in microfuge tubes. After centrifugation for 10 min at 17,000g and ambient temperature, 250  $\mu$ l of the plasma in each tube were replaced by plasma of a *g*HAT patient or an endemic control. With each blood sample, 6 volumes of 70  $\mu$ l were spotted on Whatman 4 filter paper, dried overnight at ambient temperature and stored in plastic bags with silica gel to keep the filters dry until use. As test samples for the *g*-iELISA, 8 discs of 6 mm diameter were punched out from each filter and eluted overnight in 400  $\mu$ l of iELISA sample diluent. In total, we prepared DBS with plasma from 95 *g*HAT patients from Nord Equateur Province in DRC, all positive in trypanolysis, and from 37 endemic controls from Kwilu Province in DRC, all negative in trypanolysis.