Materials and Methods

Buffers and reagents. All buffer materials unless otherwise stated were supplied by Pierce (USA) and Sigma Aldrich (USA) and used as is. MasterBeads carboxylic acid (500nm), Master Beads streptavidin (500 nm) and Storage Buffer were purchased from Ademtech (FR). Saliva and plasma samples were obtained from apparently healthy donors. The plasma was freshly prepared from blood by centrifugation for 10 min at 1500 g. cTnI-free human serum was purchased from Hytest (FI).

Functionalization of magnetic particles. For the troponin assay, carboxylated magnetic particles were coupled with antibodies according to the one-step coating procedure described by Dynal Biotech (Invitrogen). Particles were washed in 25 mM MES (2-(N-morpholino)ethanesulfonic acid) buffer, using a magnetic particle concentrator. They were resuspended in MES buffer at 10 mg/ml. Tracer anti-cTnI antibody (mAb clone A34780359P, BiosPacific, USA) was added to a final concentration of 50 µg Ab/ mg particle and incubated for 30 min under agitation. In order to activate the carboxyl groups, EDC (N-3-dimethylaminopropyl-N-ethylcarbodiimide hydrochloride) in MES was added to the particles at 2 mg/ml and incubated for 30 min at 600 rpm. Tris-HCl (final concentration 50 mM, pH 7.4) was added to inactivate the carboxyl groups, followed by an incubation of 30 min at 600 rpm. The particles were washed twice in 0.5 ml Tris-HCl. Two additional wash steps were performed with 0.5 ml of Storage Buffer (Ademtech). After resuspension in Storage Buffer at a concentration of 10 mg/mL, the particles were sonicated and stored at 4°C. Particles conjugated with antibodies to drug molecules were kindly provided by Concateno. These were prepared using a method similar to the two-step coating procedure (with N-hydroxysuccinimide) from Dynal Biotech. We checked the specificity of the antibodies in competitive immunoassays with drug mixtures; cross-reactivity was only observed between the amphetamine and meth-amphetamine antibodies.

Preparation of dry magnetic particles in the assay microchamber. Particles coated with anti-drug antibodies were resuspended in a drying buffer (10mM Tris-HCl, 10 wt% sucrose, 1 wt% BSA, pH 7.5) at 10 mg/ml, using a magnetic particle concentrator. Aggregates were
removed by two times three seconds sonification using the sonicator probe. The plastic top parts of the cartridge were made by in-house injection moulding of polystyrene and contained a cavity for the dried magnetic particles. For a single-drug assay, 150 nl of magnetic particles suspension was deposited in the cavity. For the multiplex drug panel, 200 nl of an equivolume mixture of particles in drying buffer was deposited at 30 mg/ml total particle concentration. For particles coated with tracer anti-cTnI antibody, a 500 nL volume of magnetic particles in drying buffer (PBS, 5 wt% sucrose, 5 wt% BSA) at 10 mg/ml was deposited in the cavity. Finally the particles were dried for 12 h at 4°C in a desiccated atmosphere provided by silica.

**Functionalization of the sensor surface.** Plastic sensor surfaces were formed by injection moulding of high binding ELISA microtiter plate substrate material. Spots 200 μm in diameter were inkjet printed with a sciFLEXARRAYER S5 (Scienion, DE) using a 2 nL print volume of BSA-drug solution (40 µg/ml in carbonate buffer) or anti-cTnI goat polyclonal antibody (Hytest) solution (150 µg/ml in PBS buffer). The printed substrates were dried for 3 min at 37 °C and then washed three times with 500 μl washing buffer (DoA: 0.05 wt% Tween-20, 4 wt % sucrose in PBS; cTnI: 0.05 wt % Tween-20 in PBS) to remove excess protein. Thereafter the cTnI cartridge was further blocked with a solution of 1 wt% BSA and 10 wt% sucrose in PBS for at least 1 h. Finally, excess buffer was drained and the substrates were dried in a desiccated atmosphere provided by silica for at least 12 h at 4 °C.

**Assembly and use of the cartridge.** The treated top and bottom parts of the biosensor were assembled to form a closed cartridge with an inlet and vent by using 180 μm thick double-sided adhesive tape. The tape was laser-cut to define a capillary transportation channel and a 1 μL assay chamber containing the optical sensing surface. The biosensor cartridges were stored at room temperature (DoA) or at 4 °C (cTnI) in sealed aluminum bags with silica pouches to ensure a dry environment. Upon use, a fluid sample was introduced into the cartridge via the inlet.

**Assay solutions.** Fluid samples were supplied to the cartridge as pure samples (in the case when the assay microchamber of the cartridges was provided with magnetic particles as dry reagent; see above) or as samples that were pre-mixed with magnetic particles in solution. Pure samples consisted of 100 % saliva or 100% citrate plasma. The saliva was collected with an in-house developed swab that was squeezed to obtain the sample, which was subsequently spiked with different concentrations of drugs. Plasma sample comprised a pool from 20
donors, which was then spiked with cTnI (molecular weight 26 kDa) in the form of troponin ITC complex (reference SRM(r)2921, HyTest). In the cTnI assay with pre-mixed magnetic particles, the assay solution was prepared by combining 40% human serum (in PBS) with one volume of sonicated magnetic particles diluted in 5 wt% BSA (in PBS) to a final magnetic particle concentration of 1 mg/ml. All assays were performed, unless otherwise stated, in triplicate.

**Magnetically-controlled assay.** The electromagnetic system consists of an upper and a lower magnet placed respectively above and below the cartridge. The magnets have copper windings around a 2.5 mm cobalt-iron alloy core. The distance of the sensor surface to the upper and lower magnet is 2 mm and 1 mm respectively. The indicated magnetic fields were calculated at the position of the sensor surface using the finite element simulations package COMSOL; the field simulations were validated by measurements with a giant magnetoresistance sensor probe. The magnetic actuation protocols for the different assays were:  

(i) *DoA test using 100% saliva:* The drug-spiked sample was supplied to the microchamber and incubated during 32 s with the dry magnetic-particle reagent. Thereafter the lower magnet was alternatingly powered (between 0 A/m and $10^5$ A/m) during 26 s in order to attract particles to the sensor surface for binding. In a final step, the upper magnet was powered ($2\times10^4$ A/m) to pull unbound particles away from the sensor surface. The total assay time was approximately 1 minute.  

(ii) *cTnI tests using samples pre-mixed with magnetic particles:* The solution was incubated for 30 s prior to insertion into the cartridge to allow particles to bind cTnI molecules. After insertion of approximately 10 μL of fluid into the cartridge, the particles were attracted towards the sensor surface for 225 s while the field was alternatingly switched ($3\times10^4$ A/m). This results in the binding of particles containing cTnI to the surface containing anti-cTnI antibodies. In the final step, the lower magnet was turned off and the upper magnet was powered ($2\times10^4$ A/m) to pull the unbound particles away from the sensor surface. The total assay time was approximately 5 minutes.  

(iii) *cTnI tests using 100% plasma samples:* The cTnI-spiked sample was supplied to the cartridge and incubated for 90 s with the dry magnetic-particle reagent inside the microchamber. The remaining parts of the actuation protocol were identical to that described above.

**Nanoparticle detection.** Magnetic nanoparticles at the sensor surface were detected with an evanescent field created by a collimated beam of LED light with a wavelength of 625 nm at an incident angle of 70° with respect to the normal, i.e. at an angle of 20° with respect to the
sensor surface. The reflected light passed through an imaging lens (f=7.5 mm, Anteryon) onto a CCD camera (Marlin F080B/C, Allied Vision Technologies). The signal due to the binding of nanoparticles to the sensor surface was calculated for each spot, averaging over an area of 20 pixels x 20 pixels. The signal was determined by relating the reflected light intensity to the measured light intensity prior to the binding of nanoparticles, using the formula Signal(t)=[R(0)-R(t)]/R(0), where R(0) is the reflected light intensity in absence of magnetic particles at the sensor surface and R(t) is the reflected light intensity during the assay.