Rapid simultaneous ultra-sensitive immunodetection of five bacterial toxins

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Experimental procedures used to prepare and characterize monoclonal antibodies. The photograph of the flow cell assembly on a microscope table.

Materials

In this work, we used Dulbbeco's modified Eagle's medium (DMEM), fetal calf serum (FCS), glutamine, a hypoxanthine–aminopterin–thymidine solution (HAT) (Gibco-Invitrogen, United states), incomplete Freund's adjuvant (IFA), *o*-phenylenediamine, SDS (Koch-Light LTD, Germany), polyacrylamide, 2-mercaptoethanol (Amresco, Unite States), protein-A-sepharose, Sephadex G-25M, MonoQ column (GE Healthcare, United States), polyclonal goat antibodies labeled with horseradish peroxidase to mouse immunoglobulins (Dako, Sweden), horseradish-labeled streptavidin, (BD Biosciences Pharmingen, United States), mouse immunoglobulin isotyping ELISA kit, EZ-Link-Sulfo-

NHS-LC-Biotin (sulfosuccinimidyl-6-[biotin-amidino]hexanoate) (Thermo Scientific, USA), culture plastic, and plates for EIA (Costar, United States).

Preparation of the mAbs to CT, SEA and SEB.

Monoclonal antibodies (mAbs) to cholera toxin (CT) were prepared in IBCH of RAS as described in our previous publication[1]. Monoclonal antibodies to the staphyloccal enterotoxins A and B were prepared in the Branch of IBCH of RAS following the procedure described in the previous work[2].

Preparation of the mAbs to LT and TSST.

Monoclonal antibodies to LT were prepared as described[1]. The hybridoma cells which demonstrated cross-reactivity to CT in indirect ELISA were eliminated on the stage of cloning. Cultures with level of activity to LT 15–20 times higher than the control were chosen for further growth. The mAbs to TSST were prepared as described[3]. The hybridoma clones which demonstrated cross-reactivity to other staphylococcal toxins (SEA, SEB) in indirect ELISA were eliminated. The antibodies from selected clones of the hybridomes were raised in the ascitic fluids of the BALB/c mice. The mAbs to LT were purified from the ascitic liquids using affinity chromatography on protein-A-Sepharose. The mAbs to TSST from the ascitic liquids were purified by ammonium sulphate fractionation with subsequent ion-exchange FPLC chromatography on a MonoQ column. The purity of the chromatographically purified mAbs was better than 95% judging from SDS PAGE[4].

Affinity constants of mAbs used in the assay

Indirect ELISA using the Beatty method[5] was used to calculate the affinity constants of these antibodies. The same antibodies both primary and secondary (biotinilated) were used in the ELISA and in microarray-based assays with beads detection.

Table S-1. Summary of binding constants of monoclonal antibodies with respect to their specific biotoxins.

Antibody	**Clone number**	**Affinity constant $K_{aff}\times10^{-9}$, M^{-1}**
Anti-SEA (binding)[a]	A14	0.49
Anti-SEB (detecting)[b]	A11	0.83
Anti-SEA (binding)[a]	A21	2.5

Anti-SEB (detecting)[b]	A6	0.45
Anti-TSST (binding)[a]	A5	2.0
Anti-TSST (detecting)[b]	A13	1.4
Anti-LT (binding)[a]	E11F4	0.16
Anti-LT (detecting)[b]	F5G2	1.5
Anti-CT (binding)[a]	F4F4	1.3
Anti-CT (detecting)[b]	B1F8	0.52

Notes to Table S-1

[a]Denotes that the antibody was used as primary (immobilized on the microarray)

[b]Denotes that the antibody was biotinilated and used as secondary (detecting)

Biotinilation of mAbs

Biotinilation was performed as described[6]. Briefly, 26.6 µl of a solution of the EZ-Link-Sulfo-NHS-LC-Biotin in MilliQ (10 mM) was added to 1 ml of antibody (concentration of 1-10 mg/ml in 0.5-2 ml PBS). The reaction mixture was stirred for 1 h at room temperature in the darkness. The antibody-biotin conjugate was purified from low-molecular impurities by a dialysis against PBS for 24 h.

Sandwich Immunoassays

ELISA were performed as described[7].

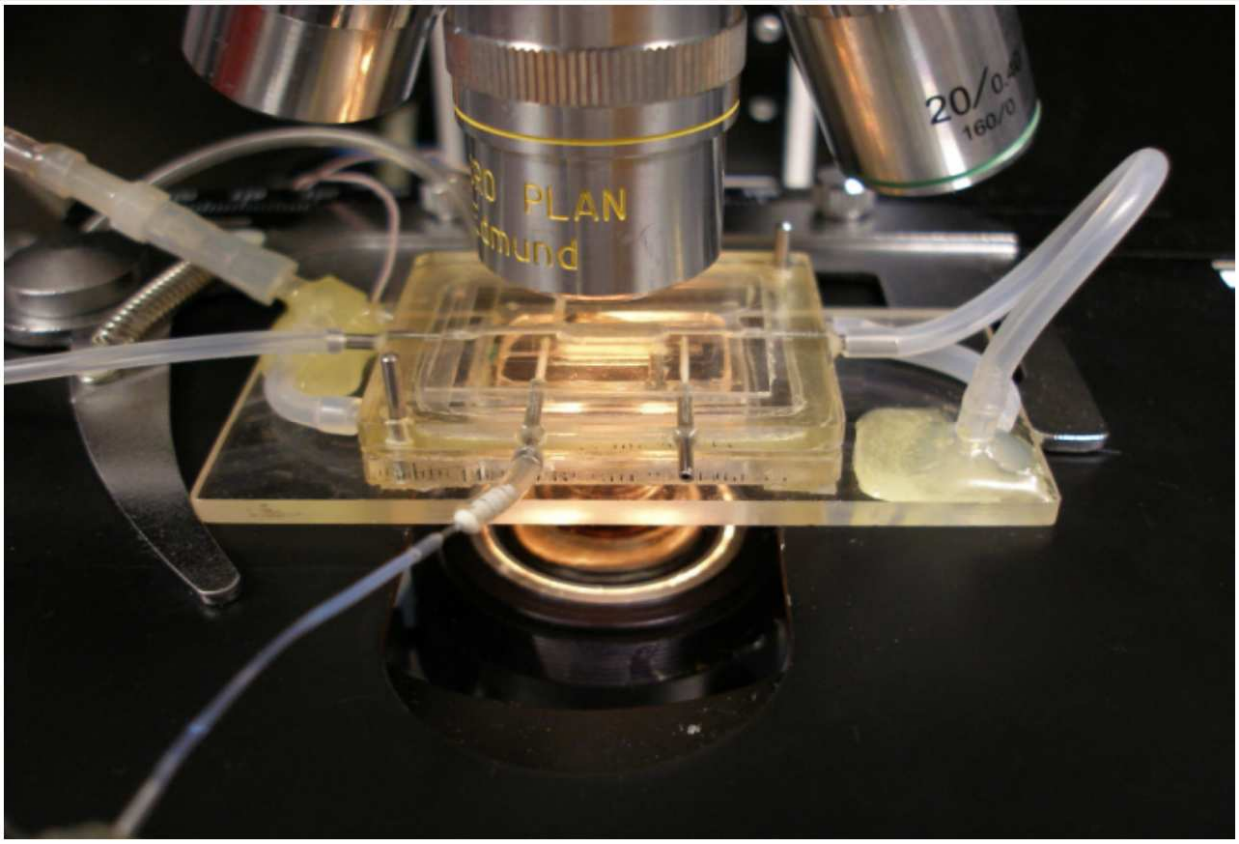

Figure S-1. The photograph of the flow cell assembly on a microscope table.

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