Supplementary materials

Affiblot: A dot blot-based screening device for selection of reliable antibody

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Table S.I: List of primary antibodies

Name	Host	Catalogue N°	Producer	Dilution
anti-chymotrypsin	Pig pAb	-	Prepared in house	1:1000 (1 µL per mL)
anti-chymotrypsin	Sheep pAb	-	Prepared in house	
anti-ApoE3	Rabbit pAb	-	Moravian Biotechnology	1:1000 (1 µg per mL)
anti-ApoE3	Mouse mAb	-	Moravian Biotechnology	
anti-EpCAM HEA 125	Mouse mAb	BK61004-N	Progen	
anti-EpCAM C10	Mouse mAb	sc-25308	Santa Cruz Biotechnology	1:1000 (1 µg per mL)
anti-EpCAM 323/A3	Mouse mAb	sc-59906	Santa Cruz Biotechnology	
anti-*Salmonella*	Rabbit pAb	0701	ViroStat	1:1000 (1 µg per mL)
anti-*Salmonella*	Mouse mAb	6321	ViroStat	
anti-*Salmonella*	Goat pAb	01-91-99	KPL	
anti-*Salmonella*	Mouse mAb	MBS531315	MyBioSource	
anti-*Salmonella*	Rabbit pAb	MBS 536004	MyBioSource	
anti-*Listeria* sp.	Rabbit pAb	4201	ViroStat	1:1000 (1 µg per mL)
anti-*L. monocytogenes*	Rabbit pAb	PA1-30487	Invitrogen	
anti-*L. monocytogenes*	Mouse mAb	3L1MabLZH1	HyTest	
anti-*E. coli*	Rabbit pAb	1001	ViroStat	1:1000 (1 µg per mL)
anti-*E. coli*	Goat pAb	PA1-30486	Invitrogen	

Table S.II: List of secondary HRP-labeled antibodies

Name	Host	Product N°	Producer	Dilution
anti-pig-IgG	Rabbit	A5670	Sigma-Aldrich	1:5000 (1 µL per mL)
anti-sheep-IgG	Rabbit	A0510	Sigma-Aldrich	
anti-mouse-IgG	Goat	A5278	Sigma-Aldrich	
anti-goat IgG	Rabbit	A5420	Sigma-Aldrich	

Well-to-well crosstalk evaluation in the affiblot

The affiblot was tested for the well-to-well crosstalk, spontaneous entry of the liquid, and overall sealing of the device. The wells were filled with methyl orange dye (Fig. S1A). The liquid did not enter the drainage channels. After switching the vacuum pump on the liquid entered the drainage channels (Fig. S1B) and the liquid was conducted to the retention chamber. Fig. S1C shows the affiblot with empty wells ready to be filled with another reagent.

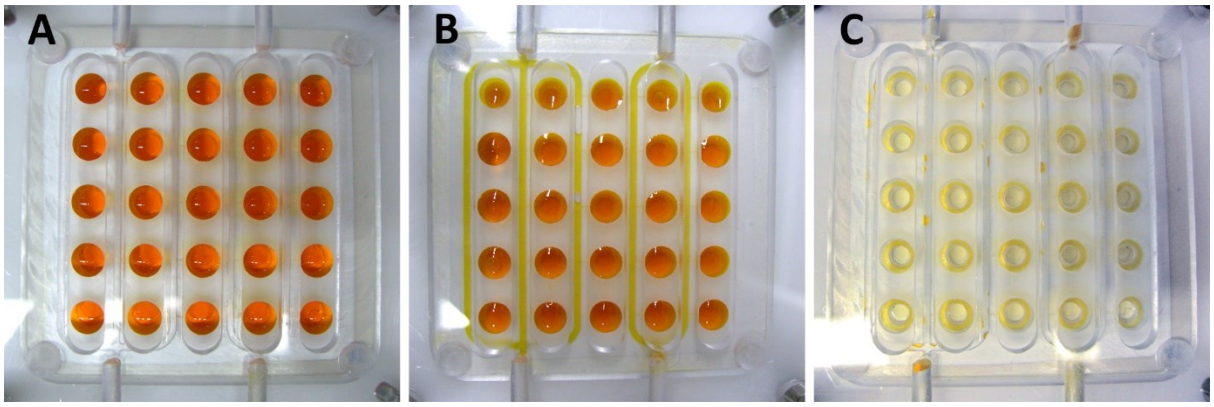

Fig. S1 Methyl orange deposition into the affiblot wells

B – beginning of liquid drain by vacuum pump, and C – liquid completely discarded.

Another crosstalk evaluation was carried out with chymotrypsin antigen and anti-chymotrypsin antibody. The antigen (25 ng per well) was applied just into two columns (III and V, Fig. S2) the other columns were filled just with the buffer. The classic dot blot technique was performed on the affiblot device. Integrated densities of spots on the PVDF membrane were evaluated by ImageLab software. The experiment confirmed that within the wells in a row there is no well-to-well crosstalk. The standard deviations of the column intensities were in the range of 3–5%. Therefore, the liquid did not pass between the wells in one row.

A

Column	I	II	III	IV	V
cAg(ng/100 mL)	0	0	25	0	25
A	13429	14148	26066	14083	25874
B	13994	14002	24490	13534	25071
C	14817	15014	23841	13712	24767
D	15114	15050	24720	14217	25278
E	15028	14811	24145	14737	23352
Average	14476	14605	24652	14056	24868
SD	657	443	768	420	840
SD (%)	5%	3%	3%	3%	3%

B

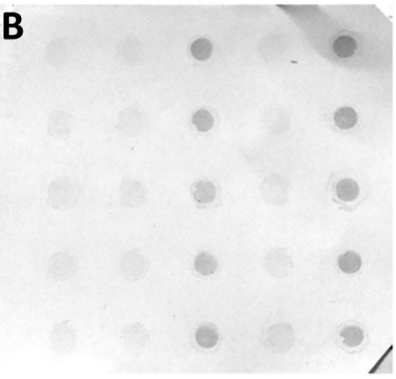

Fig. S2. Evaluation of well-to-well crosstalk carried out on affiblot.

A. The intensity of spots in columns I–V. B. Chymotrypsin, 25 ng/well, was applied in columns III and V on PVDF membrane. PBS as a blank was applied in columns I, II, and IV. Anti-chymotrypsin antibodies diluted 1:2,000 and the secondary antibody 1:8000. Standard deviation SD was below 5 %.

Evaluation of Ag-Ab model system on dot blot and ELISA

The model system used in the article was before its application in the affiblot thoroughly tested on classic dot blot and ELISA. Various concentrations of primary (1:1,000, 1:2,000, 1:3,000 and 1:5,000) and secondary antibodies (1:8,000 and 1:4,000) were optimized by dot blot technique. The chymotrypsin was applied in concentrations 0.5, 1, 2, and 3 μg mL^{-1}. Also, two negative controls (bovine serum albumin and PBS) were added to each membrane. Finally, the best results were achieved with primary antibody diluted 1:2,000, and secondary antibody diluted 1:8,000. In the next experiment, a wide range of antigen concentrations of 0.001–1 μg per 100 mL was performed on the PVDF membrane in triplicate along with the negative controls (BSA and PBS), see Fig. S3. The results showed the working range for the antibody-antigen (1–50 ng per well) and also low non-specific adsorption for the negative controls.

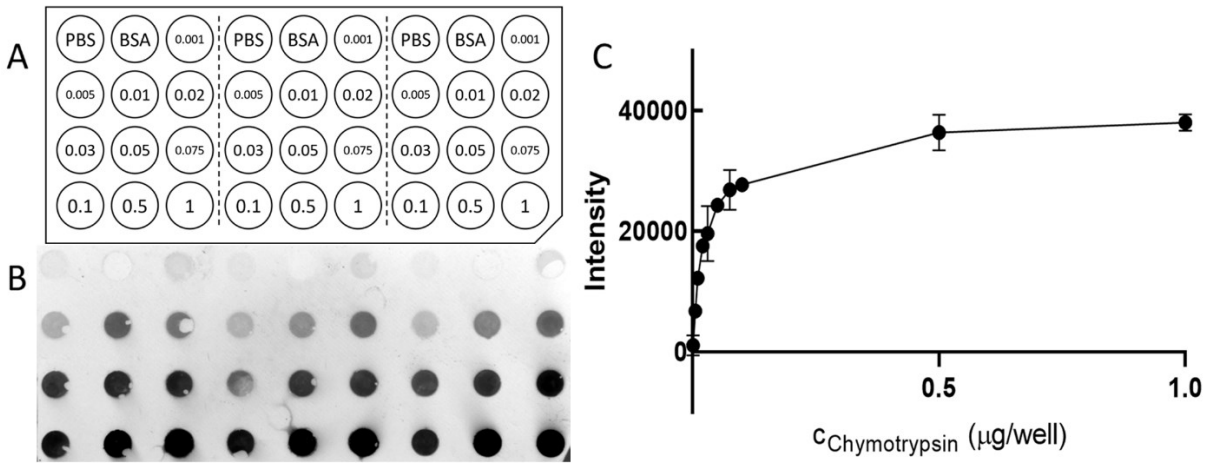

Fig. S3. Classic dot blot analysis of concentration range of chymotrypsin.

Chymotrypsin (0.001 – 1 μgper100 mL) on PVDF membrane in triplicate. A. Amount of chymotrypsin or negative control (PBS, BSA) on a membrane is mentioned in μg. B. PVDF membrane spot intensity was measured by ChemiDoc™ station (Bio-Rad Laboratories, Hercules, CA, USA). C. The plot of intensities of the membrane spots in various chymotrypsin concentrations.

Further evaluation of the concentration of chaotropic reagent (ammonium thiocyanate) was carried out on dot blot (data not shown). Various concentration ranges were applied: 0-0.5-1.5-3 M in the first attempt, 0-0.5-1-2 M in the second, and 0-0.5-1-1.5 M in the last attempt which was found the most effective for affinity/avidity evaluation of antibodies. The incubation time 5 min was preserved. Results were also confirmed by the ELISA method in the concentration range of chaotropic reagent 0-3.5 M. Results are discussed in the manuscript "Avidity evaluation by affiblot, commercial dot blot, and avidity ELISA".

Affiblot with anti-chymotrypsin antibodies

The affiblot was tested on a model system comprising chymotrypsin antigen and polyclonal anti-chymotrypsin antibody. A chymotrypsin concentration series of 0.005 µg per 100 µL, 0.01 µg per 100 µL, 0.025 µg per 100 µL, 0.05 µg per 100 µL, and 0.1 µg per 100 µL was applied on the PVDF membrane in quadruplicate. The chymotrypsin concentration increased from left to right in rows 1 and 4 and decreased from left to right in rows 2 and 5 (Fig. S4A and S4B). The wells in row 3 were filled with washing buffer and served as blanks. Hyperimmune pig serum was diluted to 1:2,000 and served as the anti-chymotrypsin IgG source. Corresponding HRP-labeled secondary antibodies were diluted with WB to 1:8,000. Classic dot blot was performed on the affiblot but the chaotropic reagent step was omitted. The intensities of the spots corresponding to the four concentrations were averaged. The relative standard deviation (SD) of the signals was < 5% except for 0.025 µg per 100 µL whose SD was 6%. No leakage between device components was observed. All of the liquid either passed to the retention chamber or drained out of the channels. The working antigen concentration range was determined by the model system to be 1–50 ng per 100 µL (Fig. S4C). The plot of the signal trend exhibited a plateau starting at ~50 ng per 100 µL. Hence, a concentration of 20 ng chymotrypsin per 100 µL was determined to be the most suitable for the subsequent experiments.

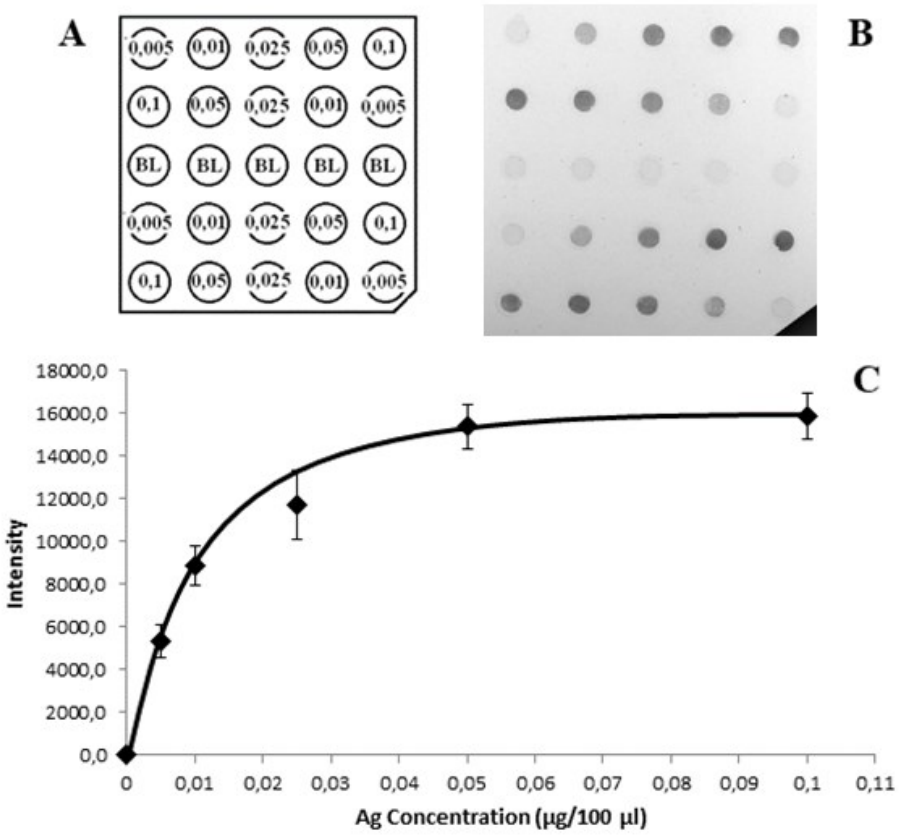

Fig. S4. Evaluation of affiblot with anti-chymotrypsin antibodies.

The chaotropic reagent step was omitted. A. Scheme of chymotrypsin application on the PVDF membrane in µg. B. The intensity of spots was measured by a ChemiDoc™ station (Bio-Rad Laboratories, Hercules, CA, USA). C. The plot of intensities of various chymotrypsin concentration vs. intensities of membrane spots. Average and SD for four replicates.

The final evaluation of affiblot was carried out with a chaotropic reagent step. The technique supposes to confirm the results from classic dot blot and ELISA, both with chaotropic reagent step. Therefore, 4 repetitions of the same experiment were accomplished with two various anti-chymotrypsin polyclonal antibodies (pig and sheep). The chymotrypsin was applied in rows 1, 2, 4, and 5 with 20 ng per well. Row 3 was reserved for blanks. Row 1 and 4 were incubated with the pig serum (1:2,000) and rows 2 and 5 with the sheep serum (1:2,000). The secondary antibodies anti-sheep and anti-pig were diluted 1:8,000. The chaotropic reagent step took 5 min and was performed in the concentration range 0-1.5 mol L^{-1} for each column. Fig. S5 shows the results of one of the PVDF membranes where intensity decrease due to the action of the chaotropic reagent.

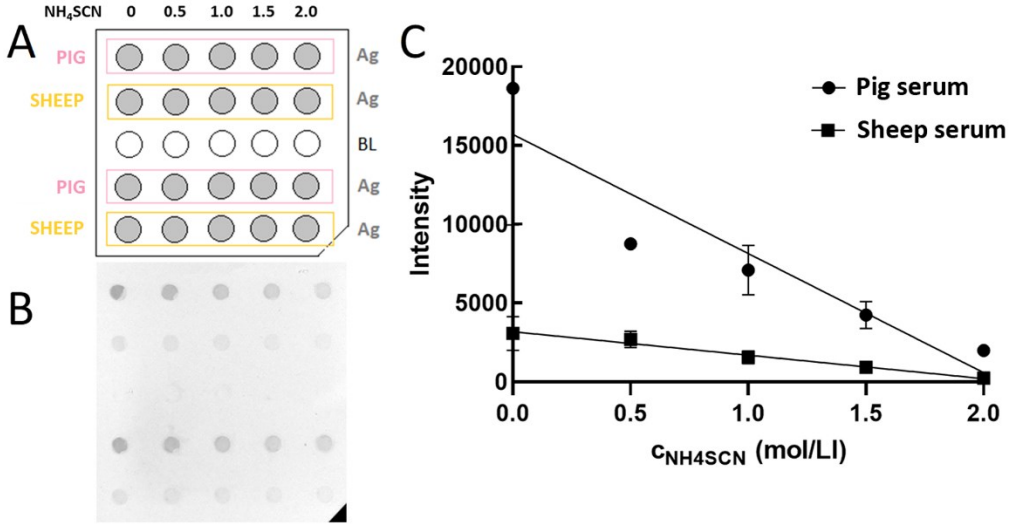

Fig. S5. Affiblot evaluation of avidity of two polyclonal anti-chymotrypsin antibodies
Hyperimmune sheep and pig sera. A. Amount of chymotrypsin on the membrane in µg. B. The intensity of PVDF membrane spots was measured by a ChemiDoc™ station (Bio-Rad Laboratories, Hercules, CA, USA). C. The plot of intensities of various chymotrypsin concentrations vs. intensities of membrane spots. Average and SD for four replicates.