Supporting Information

Ultrasensitive Rapid Detection of Human Serum Antibody Biomarkers by Biomarker-Capturing Viral Nanofibers

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Production of Polyclonal Anti-Sap2-IgG in Rabbits to Be Used for Determining Detection Limit

1. Rabbit Immunization and Serum-Collection. Serum was collected from Sap2-immunized rabbits by following a standard protocol.[1, 2] Two adult female New Zealand white rabbits were used for the production of polyclonal anti-Sap2-IgG. Briefly, on day 1, both rabbits were immunized with 1 mg of purified Sap2 protein emulsified with complete Freund's adjuvant (Sigma, St. Louis, MO) through subcutaneous injections at 10 different sites. On day 21, the rabbits were booster-immunized with Sap2 protein (500 μg each rabbit) mixed with incomplete Freund's adjuvant. 10 days after the booster-immunization (on day 31), the blood from each animal was harvested and the serum was stored at -80℃. The blood samples collected before immunization and on day 21 (after booster immunization) were also collected as negative controls.

2. Purification and Characterization of Anti-Sap2-IgG. Anti-Sap2-IgG was purified with Protein G (GE Healthcare Life Science) by following the manufacturer's protocol. The purified anti-Sap2-IgG was used at a concentration of 6.41 mg/ml. Then the specificity of the anti-Sap2-IgG was tested by ELISA and Western-blotting assay: **(1) ELISA**. The purified anti-Sap2-IgG solution was diluted serially (1:12800, 1:25600, 1:51200, 1:102400, 1:204800, 1:409600, 1:819200 and 1:1638400) and tested by ELISA. Specifically, 96-well immunoplates were coated with 100 μl of purified Sap 2 protein (3 mg/l) in 50 mmol/l carbonate buffer (pH9.6) overnight at 4℃ and blocked with 200 μl PBS with Tween® 20 (PBST) containing 1% bovine serum albumin (BSA) for 2 h at 37℃. Then anti-Sap2-IgG solution with various dilutions was added into the wells of the plate, respectively, and allowed for incubation for 1 h at 37℃. After

incubation, each well of the plate was washed and incubated with 100 μl HRP-conjugated goat-anti-rabbit IgG (dilution with a ratio of 1:5000 in PBST) for 1 h at 37℃. Finally, each well of the plates was washed with PBST and incubated with 100 μl TMB solution for 20 min at 37℃. The absorbance of the yellow HRP-converted substrate was measured at 450 nm using a microplate reader (Figure S2). The results indicated that the titer of anti-Sap2-IgG was 1: 409600. **(2) Western Blot assay.** The Sap2 protein was run on a 10% SDS-PAGE gel, which was then transferred to mitrocellulose membranes (Bio-Rad) and blocked with 5% non-fat milk in TBST overnight at 4℃. The next day, the membrane was incubated with purified anti-Sap2 solution (6.38 mg/ml) for 1 h at 37℃, washed with PBST, exposed to HRP-conjugated goat-anti-rabbit IgG for 45 min at room temperature, and finally stained with 2-amino-9-ethylcarbozole (Amresco, USA) as a chromogen. The non-immunized serum was used as negative control. The results confirmed the specificity of our purified anti-Sap2-IgG (Figure S3).

Determination of the Limit of Detecting anti-Sap2-IgG by Using Sap2 Protein and Our ASIT-MNP-phage as Biomarker-Capturing Probes in ELISA

1. Sap2 Protein-based ELISA Method. A 96-well plate was coated with 100 μl Sap2 protein (3μg/ml) in carbonate buffer (pH 9.6) overnight at 4℃. Next day, each well of the plate was washed 3 times with PBST and blocked with 200 μl PBST containing 1% BSA for 2 h at 37℃, followed by incubation with 100 μl of anti-Sap2-IgG with serial dilutions (ranging from 0.03125 ng/ml to 500 ng/ml). After incubation for 1 h at 37℃, the plate was washed and incubated with 100 μl HRP-conjugated goat-anti-rabbit IgG (dilution 1:5000 in PBST) for 1 h at 37℃. Finally, each well of the plate was washed with PBST and incubated with 100 μl TMB solution for 20

min at 37℃. The absorbance at 450 nm of each well was measured using a microplate reader and plotted against the concentration of the corresponding diluted anti-Sap2-IgG solution (Figure 3b). A good linear relationship between ODs and concentrations of anti-IgG was established in the concentration range of 0.5 ng/ml-70 ng/ml ($y=0.0101x+0.1459$, $R^2=0.9957$). The analysis of the data shown in Figure 3b using a standard analytical chemical method[3, 4] showed that the limit of detection is approximately 89.56 pg/ml.

2. ASIT-MNP-phage-based ELISA Method. 10 ml of each of the diluted anti-Sap2-IgG solution ranging from 1.25 pg/ml to 2.5 ng/ml was incubated with the ASIT-MNP-phage complexes for 1 h. Then the ASIT-MNP-phage complexes were collected and enriched by a magnet and the captured anti-Sap2-IgG was eluted off from ASIT-MNP-phage using 100 µl of an elution buffer. The eluted anti-Sap2-IgG was tested by ELISA with the same protocol described in the last section. The results (Figure 3c) indicate that a good relationship between ODs and concentrations of IgG could be established in the concentration range of 5 pg/ml-200 pg/ml ($y=0.0012x + 0.1156$, $R^2=0.9997$). The analysis of the data shown in Figure 3c using a standard analytical chemical method[3, 4] showed that the limit of detection is approximately 1.118 pg/ml.

Table S1. After the last round of selection, the eluted phage colonies were randomly picked up for sequencing and occurrence number for each colony was counted.

LRTSPSKQRDHLTSP[a)]	4[b)]	FQDQHLLSPLPTLDA	1	NRCGPLKSQFRMPGS	1
IRQTRSRTRLSRWAS	4	LLRQFSFQHPRPLLR	1	TISLVTWPREPQLQT	1
PTYSLVPRLATQPFK	3	CRPYRRSLRS RLLPW	1	QPITGCYLQTAFPPH	1
PSATERLPAQSHPEG	2	AAIAPNMLTSMRDQL	1	LLIPPHQSPLLADQI	1
TVSDEVRLLRLPSTA	2	RYTLLLQTSTLPQRR	1	QFYRPMNLSLHFMPA	1
LALSPQSWPGPANSA	2	PPVINVGSTLPSQDA	1	PYSPPPPPQVRALQH	1
PFISYGAQTPLLPVF	2	HPTLFLPPHLPVPSL	1	SFQPDQLFHLLRAAY	1
TWVASALKNLLYACP	2	LPRIPMSQLVVLQWF	1	HSLLPNAKHPSAPPV	1
QLPSSTPLYATTWQP	2	PADNTNTRKSTPHQM	1	VKTSPPPLTHLLLEH	1
TPPSSSLVVLQSKAT	2	QMPDNWSSNSYSQPP	1	WDLVLVRWKQCESHI	1
NNPSEPRVLLSLYQS	1	IVIQTNKSHLPAAKA	1	DQSQAPFKPEKAYSM	1
QPSIRSHPSLMAEIL	1	PPAPSYARSSRTSSH	1	APPHQAQRRSVPVVA	1
APSCFEGPQLVTPPR	1	TLQWVPAVPPQSVWA	1	LTQQLRLPSPGHGAL	1
DWRHSRLDLWPRNMV	1	ATSREFSQTMTNSSL	1	EQHVMVVHVSRPALD	1
PWSPLDLPWSLSRLH	1	LSLPQSGLPRSALRG	1	QQAPVYMFEPLSWCT	1
TVDLSRASKYSSTLD	1	PYKPQLSHSTHMRQS	1	--------------	-

[a)] amino acid sequence of each peptide displayed on phage clones screened by phage library;

[b)] occurrence frequency of each peptide

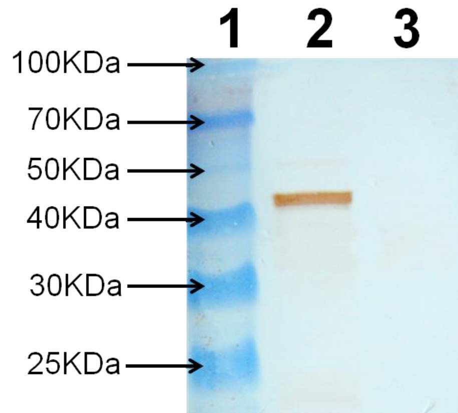

Figure S1. Western blot for PK-ASIT phage and wild-type (WT) phage with candidiasis serum. Left lane, maker; middle lane, PK-ASIT phage; and right lane 3, WT phage. The following experimental procedures were carried out to obtain the data. First, PK-ASIT phage and wild-type phage were run on a SDS-PAGE gel and then transferred onto one nitrocellulose membrane at the same time. Second, the membrane was incubated first with serum collected from candidiasis patients and subsequently with HRP-conjugated goat-anti-human IgG. Third, the membrane was stained with 2-amino-9-ethylcarbozole.

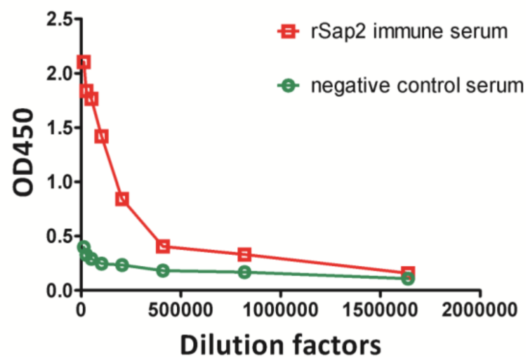

Figure S2. ELISA result of the purified anti-Sap2-IgG solutions with a series of dilutions.

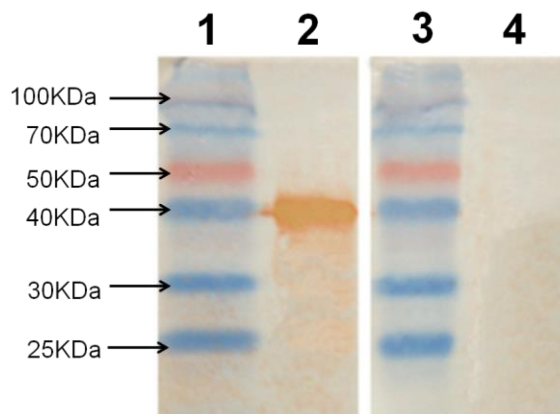

Figure S3. Western blotting analysis of anti-Sap2-lgG. Left image: Lane 1, marker; Lane 2, anti-Sap2-IgG. Right image: Lane 3, marker; Lane 4, non-immunized serum.

References:

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