

Supplementary Information

Dual Lateral Flow Assay Using Quantum Nanobeads for Quantitative Detection of BDNF and TNF- α in Tears

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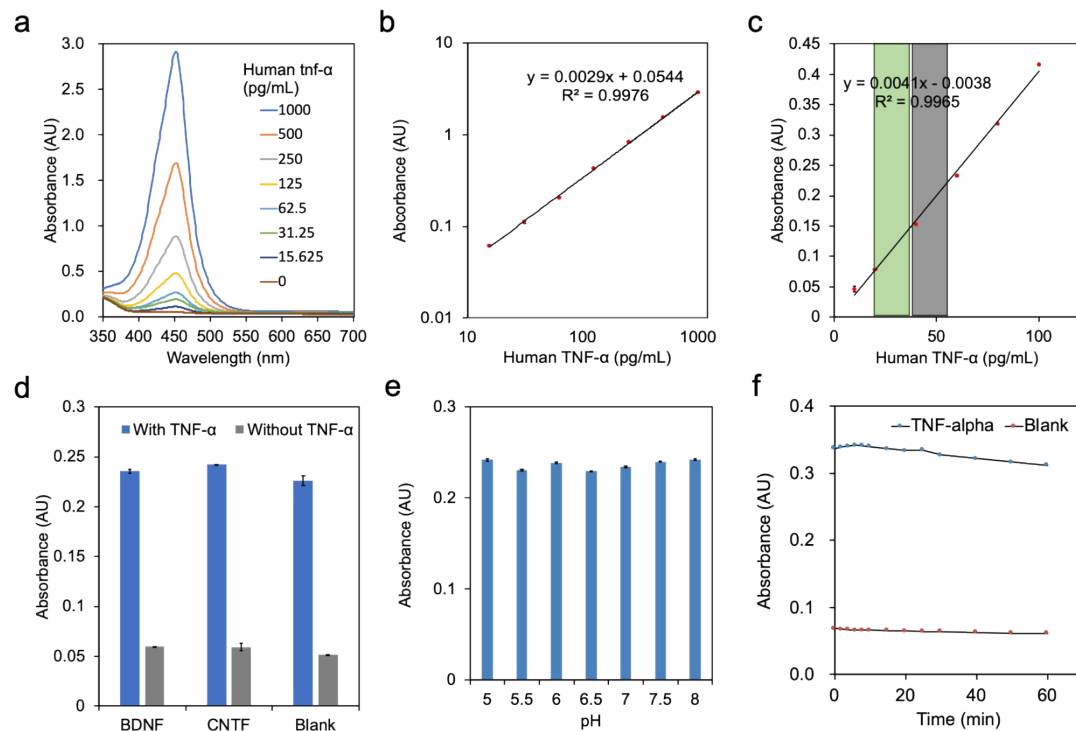


Figure S1 ELISA test for TNF- α detection a. The absorbance spectrum of the TNF- α ELISA test with TRIS buffer as diluent buffer. b. The standard curve of TNF- α ELISA kit with R^2 0.999. c. The standard curve ranges from 0 to 100 ng mL⁻¹ of CNTF ELISA kit with R^2 0.997. The grey area represents the range of TNF- α in tear fluid from glaucoma patients and the green area represents the tear TNF- α level range from control subjects. d. The selectivity of the human TNF- α ELISA kit. e. The pH dependence test for the human TNF- α ELISA kit. The pH value ranged from 5.0 to 8.0. f. The absorbance reading of TNF- α and blank sample after certain time.

Table S1 Summary of studies to investigate the level of TNF- α and BDNF in body fluid of glaucoma patients.

Study	Body fluid	Subjects	Detection method	Level in patient (pg·mL ⁻¹)	Level in control (pg·mL ⁻¹)	P Value
Shpak et al ¹	Tear	55 patients with POAG*	ELISA	78.0 $\pm$ 25.1	116.2 $\pm$ 43.1	<0.001
	Aqueous humor	29 healthy controls		35.2 $\pm$ 14.2	54.6 $\pm$ 29.6	<0.001
	Blood	27 patients with POAG*		19230 $\pm$ 5960	22440 $\pm$ 7580	0.016
Burgos-Blasco et al ²	Tear	29 healthy controls	Multiplex immunobead assay	42.7 $\pm$ 7.1	28.6 $\pm$ 5.5	0.094
Çomoğlu et al ³	Tear	17 healthy controls (18 patients with PD*)	Multiplex immunobead assay	-	110.7 $\pm$ 87.2	
Ionescu et al ⁴	Tear	18 healthy controls (30 patients with KC*)	Multiplex immunobead assay	-	108.1 $\pm$ 15.8	

POAG: Primary open angle glaucoma PD: Parkinson's disease KC: Keratoconus

Table S2 Comparison between commercial ELISA for TNF- α and BDNF and dual-test

LFA strip

	ELISA		LFA	
	TNF- α	BDNF	TNF- α	BDNF
Response time	90 mins	90 mins	30-40 mins	30-40 mins
Detection limit	4.32 pg·mL ⁻¹	2.40 pg·mL ⁻¹	3.39 pg·mL ⁻¹	4.13 pg·mL ⁻¹
Intra-assay CV%	2.5%	2.8%	5.3%	6.1%
Inter-assay CV%	3.1%	5.3%	6.6%	8.3%
Recovery	80%-113%	85% - 112%	105.9%-112.5%	95.1%-100.5%
Selectivity	High	High	High	High
pH affects	Low	Low	Low	Low

CV: Coefficient of Variation;

Table S3 Comparison of different fluorescence based LFA and detection methods

Study	Biomarkers	Target	Label	Detection method	LOD
This work	TNF- α	Tear fluid	QBs	Smartphone	3.39 pg·mL ⁻¹
	BDNF			camera+3D printed readout box	4.13 pg·mL ⁻¹
Yang et al ⁵	CRP PCT	Serum	SiO ₂ @QDs	FIC-S1 fluorescent strip reader	0.5 ng·mL ⁻¹ 0.05 ng·mL ⁻¹
Wang et al ⁶	SARS-CoV-2 antigen	Throat swab samples	SiTQD QBs	Commercial fluorescent reader	5 pg·mL ⁻¹
	FluA H1N1				50 pfu·mL ⁻¹
Yang et al ⁷	SARS-CoV-2 S1 protein	Throat swab samples	Dual-signal nanocomposite	Commercial fluorescent reader	18.3 pg·mL ⁻¹
	SARS-CoV-2 N protein				2.2 pg·mL ⁻¹
Wang et al ⁸	SARS-CoV-2 N gene	Throat swab samples	Commercial QBs	Handheld dual-color fluorescent test strip reader	1.90 copies· μ L ⁻¹
	SARS-CoV-2 ORF1ab gene				6.07 copies· μ L ⁻¹
Mahmoud et al ⁹	Interleukin-6	Serum	Quantum dot, aptamer	Smartphone	100 pM
	Thrombin			camera+3D printed readout box	3 nM
Rong et al ¹⁰	Zika virus nonstructural protein 1	Buffer	Quantum dot	Smartphone	45 pg·mL ⁻¹
		Serum		camera+3D printed readout box	150 pg·mL ⁻¹

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