Supplementary materials

Functionalized graphene oxide-antibody conjugate-based electrochemical immunosensor to detect *Opisthorchis viverrini* antigen in urine

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Indirect ELISA measurement

Monoclonal OV antibody (mAb, IgM, KKU 505) 100 µL was coated on 96-well microtiter plates and incubated overnight at 4°C followed by washing with PBST. The plates were blocked with 200 µL of 5% skimmed milk solution and incubated at 37°C for 1 hr. The plates were then washed for 5 times with PBST. Urine samples were centrifuged at 1500 rpm for 5 min at 4°C. The centrifuged urine sample (100 µl/well) was added into the plates and incubated at 37°C for 2 hr. The second polyclonal anti-rabbit IgG antibody (100 µl) was added and incubated at 37°C for 1 hr. The plates were washed 3 times with PBST between each immobilization steps. Biotin conjugate (100 µl/well) was incubated at 37°C for 1 hr. Then, streptavidin horseradish peroxidase (HRP) conjugate (100 µl/well) was added. After 30 min incubation, the plates were washed again with PBST. The substrate Orthophenylenediamine hydrochloride was added to wells and incubated for 20 min at room temperature. The reaction was stopped by the addition of 2M sulfuric acid (H_2SO_4) and the absorbance was measured at 492 nm.

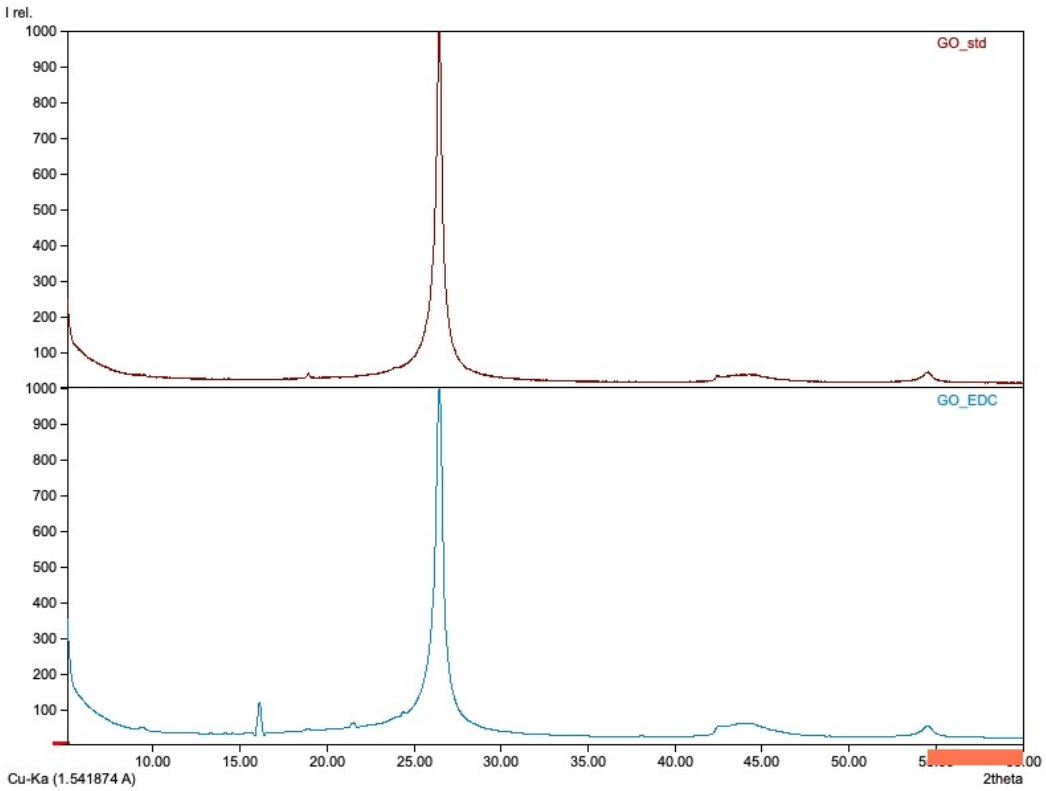

Figure S1 XRD patterns of original GO and EDC/NHS functionalized GO

Table S1. OV positive percentage based on age and gender by sensor detection

Sample	**Method**	**Sex**	**Age**		**Interval of Age**				
		Male (OV positive, %)	**Female (OV positive, %)**	**Mean ± SD**	**< 30**	**31-40**	**41-50**	**51-60**	**>60**
Group 1		50	97	50.56 ± 18.69	7	7	30	44	59
	Sensor	(38,76)	(70,72)		(7,100)	(3,42.8)	(23, 76.3)	(30,68.2)	(45,76.3)
	ELISA	(36,72)	(62,63.9)		(7,100)	(3,42.8)	(23,76.3)	(31,70.5)	(46,78.0)
Group 2		39	91	50.40 ± 16.97	10	13	29	46	32
	Sensor	(18,46.2)	(36, 39.6)		(3,30)	(5,38.5)	(14,48.3)	(22,47.8)	(10,31.2)
	OV-RDt	(15,38.5)	(35,38.5)		(3,30)	(6,46.2)	(14,48.3)	(18,39.1)	(9,28.1)
Group 3		68	170	42.9 ± 19.83	70	11	50	77	45
	Sensor	(25,37.3)	(54,32.1)		(9,12.9)	(4,36.4)	(19,38)	(23,29.9)	(24,53.3)
	ELISA & OV-RDT	(17,25)	(38,22.4)		(5,7.14)	(3,27.3)	(14,28)	(16,20.7)	(16,35.5)

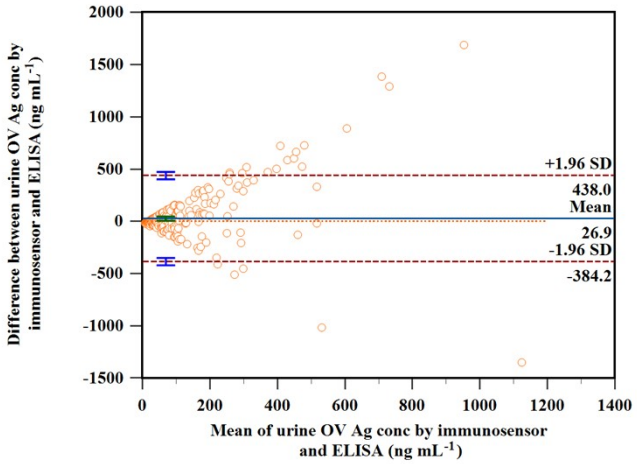

Figure S3 The Bland-Altman plot for concordance analysis between the electrochemical immunosensor and conventional ELISA techniques. The solid line illustrates the mean of absolute difference between the methods. The dashed line 95% limit of agreement (n = 400).

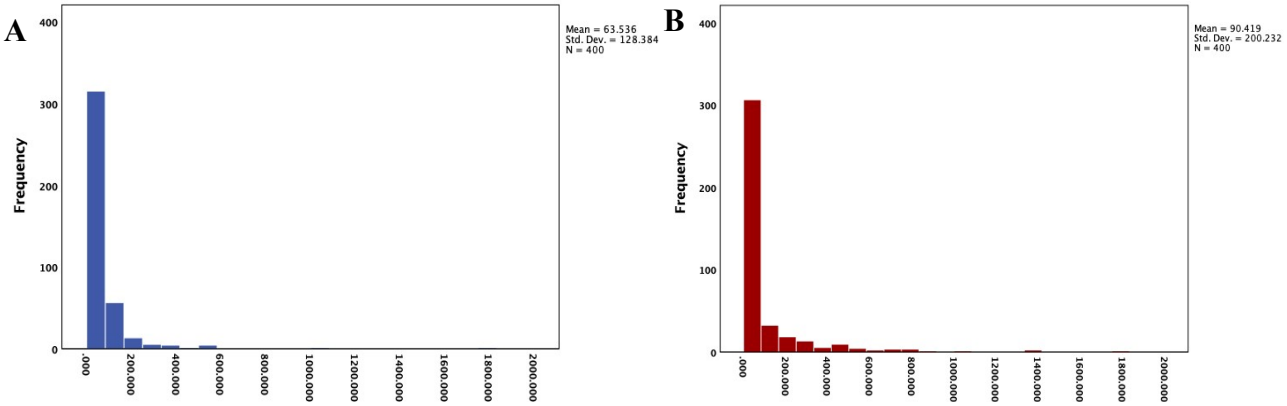

Figure S3 Frequency distribution of urine OV Ag concentration measured by (A) ELISA and (B) immunosensor