Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

SUPPORTING INFORMATION

Rapid diagnosis of egg allergy by targeting ovalbumin specific IgE and IgG4 in serum at a disposable electrochemical immunoplatform

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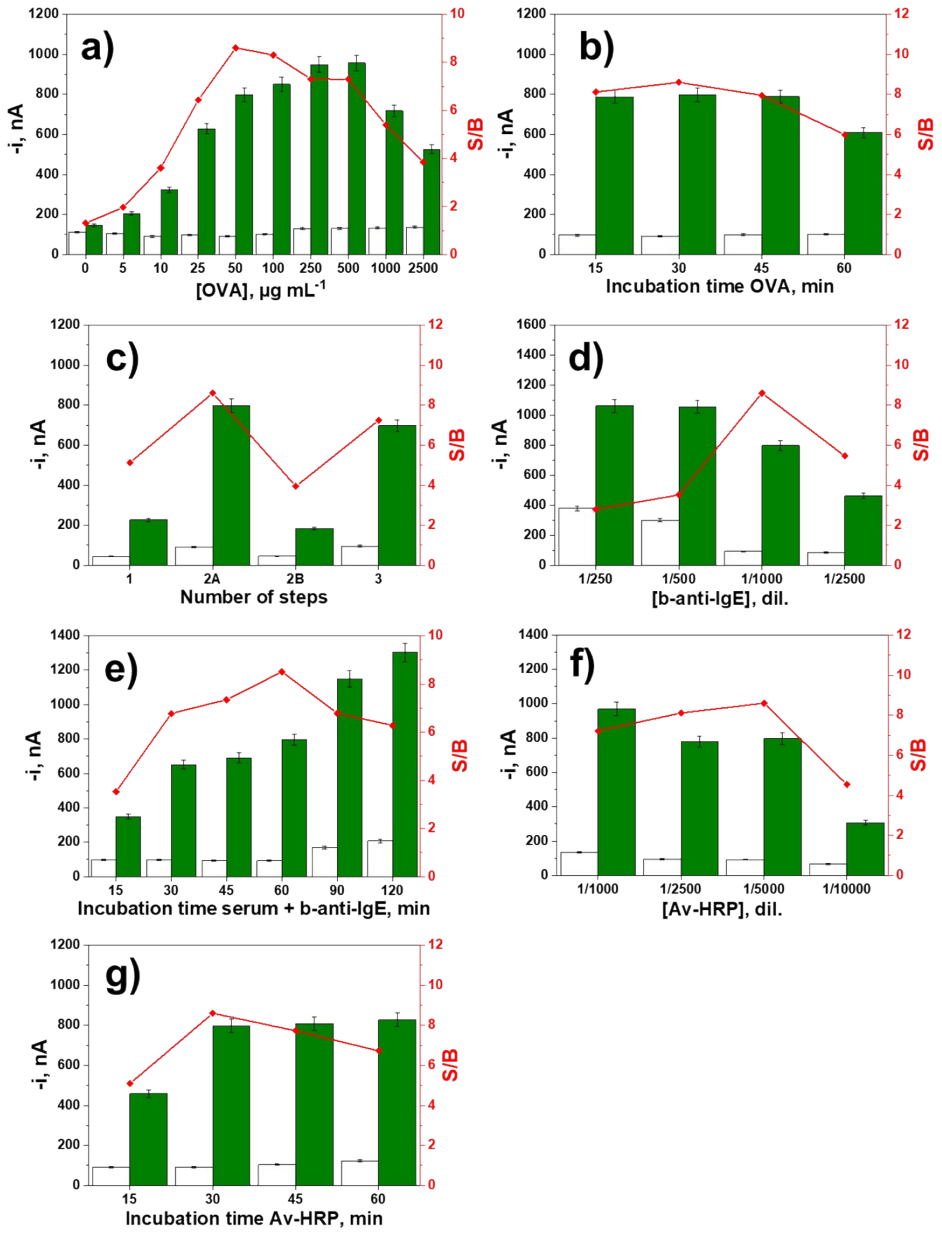

Fig. S1 Optimization of key experimental variables involved in the preparation and functioning of the bioplatform prepared for the amperometric determination of OVA-specific IgE. Dependence of the amperometric responses measured in the absence (empty bars, B) and in the presence of 0.30 kU L^{-1} (full bars, S) IgE serum standard solutions, and the corresponding S/B ratios (in red), with OVA concentration and incubation time (a, b); number of steps involved in the preparation of the Av-HRP-b-anti-IgE-IgE-OVA-MBs (c); b-anti-IgE dilution (d) incubation time with IgE serum standard and b-anti-IgE mixture solutions (e); and Av-HRP concentration and incubation time (f, g).

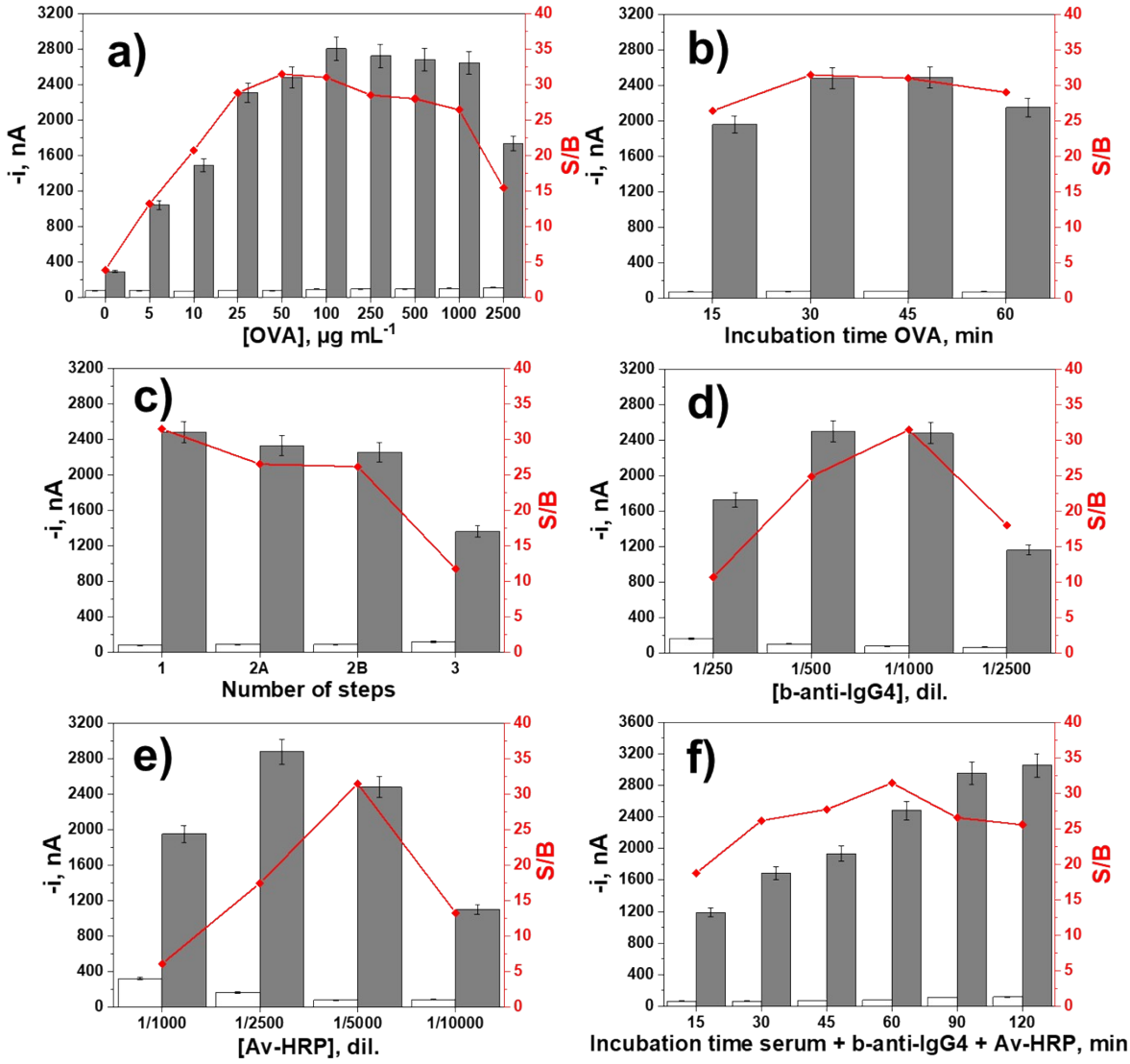

Fig. S2 Optimization of key experimental variables involved in the preparation and functioning of the bioplatform prepared for the amperometric determination of OVA-specific IgG4. Dependence of the amperometric responses measured in the absence (empty bars, B) and in the presence of 0.036 µg L^{-1} (full bars, S) IgG4 serum standard solutions, and the corresponding S/B ratios (in red), with OVA concentration and incubation time (a, b); number of steps involved in the preparation of Av-HRP-b-anti-IgG4-IgG4-OVA-MBs (c); b-anti-IgG4 and Av-HRP concentrations (d, e), and incubation time with IgG4 serum standard, b-anti-IgG4 and Av-HRP mixture solution (f).

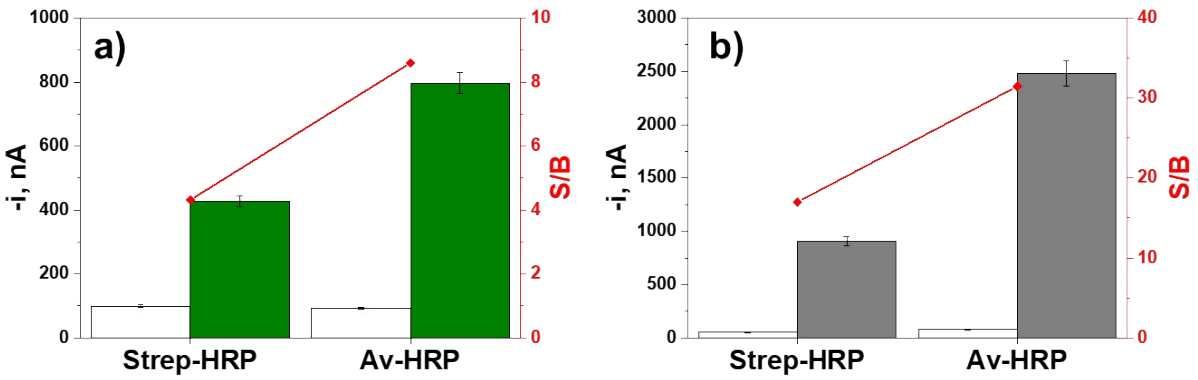

Fig. S3 Amperometric responses (and corresponding S/B ratios) measured with the prepared bioplatforms for the single determination of IgE (a) or IgG4 (b) in the absence (empty bars, B) and in the presence of 0.30 kU L^{-1} IgE or 0.036 µg L^{-1} IgG4 (full bars, S) serum standard solutions using enzyme labelling of the magnetic bioconjugates with Strep-HRP or Av-HRP.

Table S1 Optimization of key experimental variables involved in the single amperometric determination of OVA-specific IgE and IgG4 with the developed bioplatforms

Experimental variable	Selected value	
	IgE	IgG4
[OVA], µg mL^{-1}	50	50
Incubation time with OVA, min	30	30
Number of incubation steps	2	1
b-anti-IgE, dil.	1/1,000	--
Incubation time with (IgE serum standard + b-anti-IgE) mixture solution, min	60	--
Av-HRP, dil.	1/5,000	--
Incubation time with Av-HRP, min	30	--
b-anti-IgG4, dil.	--	1/1,000
Av-HRP, dil.	--	1/5,000
Incubation time with (IgG4 standard + b-anti-IgG4 + Av-HRP) mixture solution, min	--	60

Table S2 Comparison of the available methodologies to determine IgE and/or IgG4 in serum

Basis	Detection type	Target Igs	LOD	Assay time	Ref.*
ELISA	Absorbance	IgG4	--	4 h	[22]
LICA based on nanomicrospheres	Chemiluminescence	Children's milk protein-specific-IgE	--	3 h	[23]
ImmunoCAP	Fluorescence	Hen's egg white-specific IgE	0.1 kU L^{-1} IgE (human serum); 0.0007 µg L^{-1} IgG4 (plasma)	1 h 40 min	[24,25]
Microarray-based method	Fluorescence	Egg-white-specific IgE and IgG4	0.25 kU L^{-1} IgE; 100 µg L^{-1} IgG4	21 h 30 min	[42]
ELISA methodologies involving the same immunoreagents as the bioplatforms	Absorbance	OVA specific IgE and IgG4	0.03 kU L^{-1} IgE; 0.006 µg L^{-1} IgG4	4 h	This work
Immunoassays performed on the surface of OVA-MBs	Electrochemical	OVA specific IgE and IgG4	LOD: 0.003 kU L^{-1} IgE; 0.0002 µg L^{-1} IgG4	1 h 30 min	This work

LICA: light-initiated chemiluminescent assay; MBs: magnetic beads; OVA: ovalbumin

** These references correspond to those given in the manuscript*

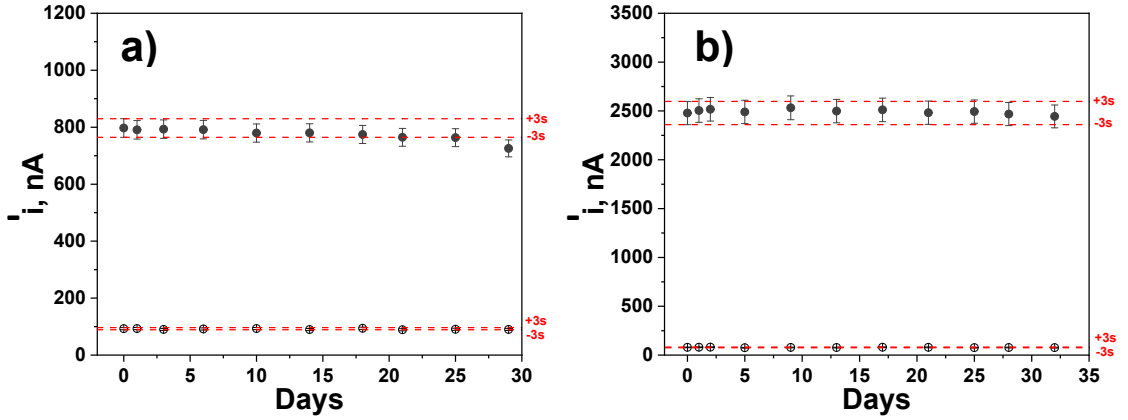

Fig. S4 Amperometric responses provided with the bioplatforms prepared from the stored OVA-MBs (at 4 ºC in filtered PBS pH 7.4) in the absence (empty circles) and in the presence of 0.30 kU L^{-1} IgE (a) and 0.036 µg L^{-1} IgG4 (b) serum standard solutions (full circles). Control limits (red dashed lines) were calculated as ± 3×mean values of three amperometric responses obtained the day of OVA-MBs preparation (day 0).

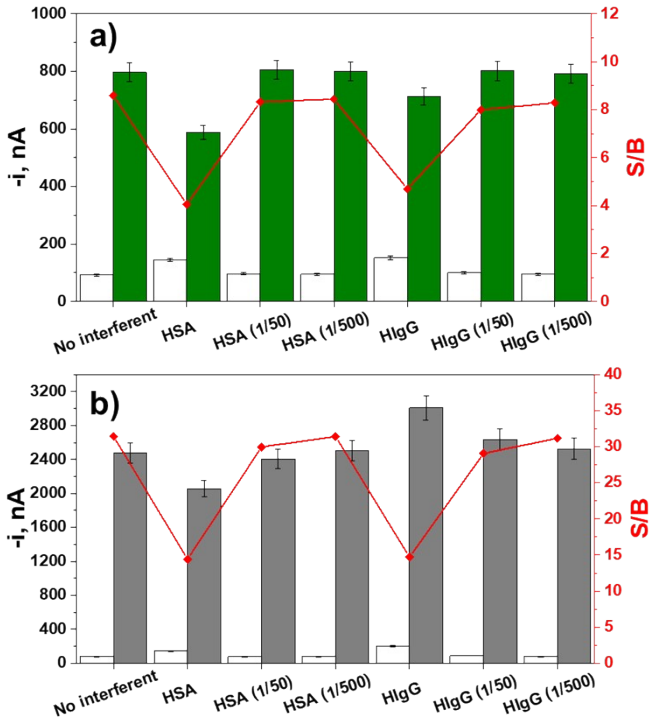

Fig. S5 Amperometric responses (and corresponding S/B ratios) provided by the developed bioplatforms for the determination of OVA-specific IgE (a) and IgG4 (b) for 0 (white bars, B), and 0.30 kU L^{-1} IgE or 0.036 µg L^{-1} IgG4 (full bars, S) serum standard solutions prepared in the absence and in the presence of 50 mg mL^{-1} HSA and 1 mg mL^{-1} HIgG undiluted or 50 and 500 times diluted.

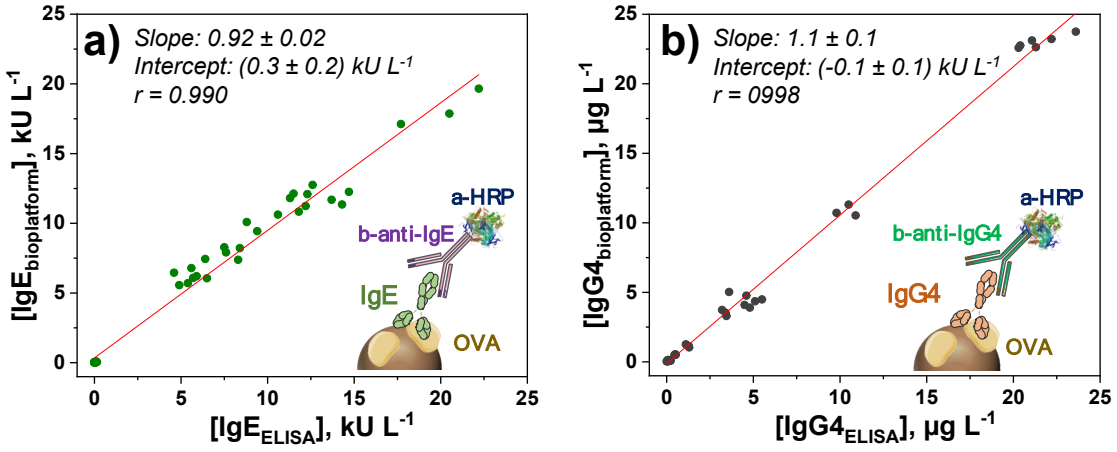

Fig. S6 Correlation plots for the concentrations of OVA-specific IgE (a) and IgG4 (b) in the analysed serum samples (replicates included in the plot) obtained with the developed electrochemical bioplatforms and the ELISA method. Red lines: regression lines with r = Pearson regression coefficient.