

Supplementary Material

Tailor-Made Immunochromatographic Test for the Detection of Multiple 17 α -Methylated Anabolics in Dietary Supplements

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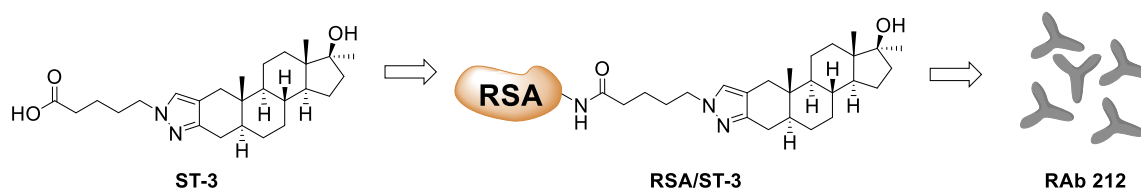


Figure 1. Chemical structures of stanazolol hapten (**ST-3**) and its conjugate with RSA (**RSA/ST-3**). By immunization of rabbit, group selective polyclonal antibodies (**RAb 212**) were obtained¹.

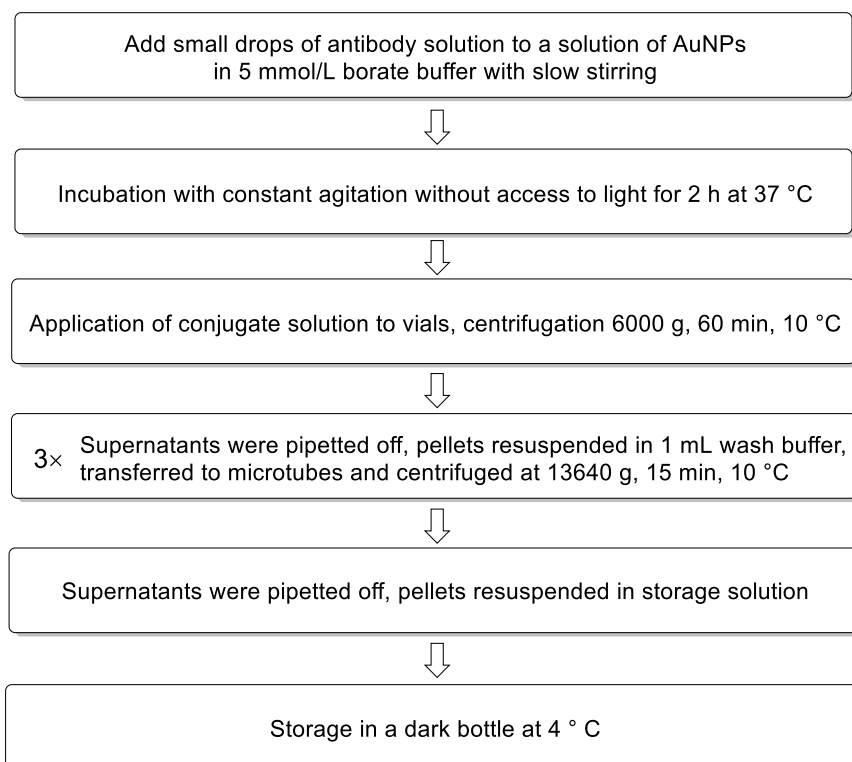


Figure S2. Procedure for the preparation of colloidal RAb 212/AuNPs solution.

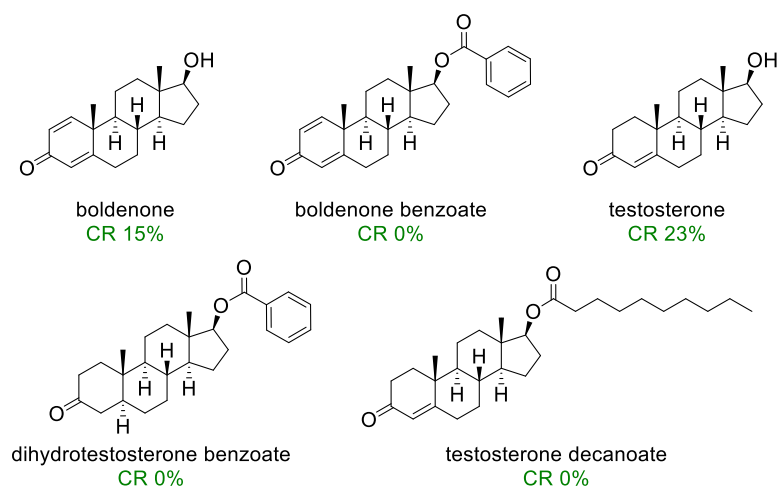


Figure S3. Tested steroids with low CR.

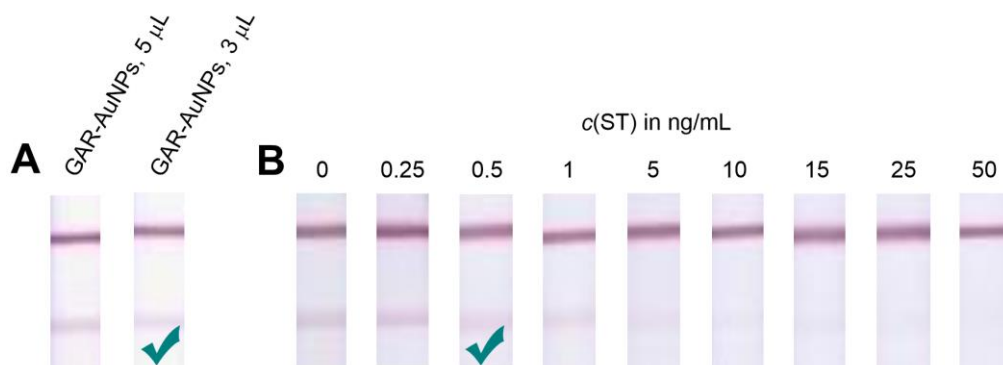


Figure S4. Selection of the applied amount of GAR-AuNPs on panel A and the calibration series for indirect open format (3 μ L GAR-AuNPs were applied) on panel B.

Table S1. Original and newly selected test materials for immunochromatographic test (closed format), including criteria in which the individual materials differed.

Membrane			Manufacturer / supplier
designation	pore size [μm]	note	
AE 98	5	selected	Whatman GmbH, Dassel, Germany
AE 99	8	tested	Whatman GmbH, Dassel, Germany
AE 100	12	tested	Whatman GmbH, Dassel, Germany
PRIMA 85	8	tested	Whatman GmbH, Dassel, Germany
PRIMA 125	12	tested	Whatman GmbH, Dassel, Germany
FF80HP	not specified	tested	Whatman GmbH, Dassel, Germany
FF120HP	not specified	tested	Whatman GmbH, Dassel, Germany
HFB13504	8	tested	Millipore Corp., Massachusetts, USA
HFB135UB	8	tested	Millipore Corp., Massachusetts, USA
Membrane pad			
designation	material	note	
HF000MC100	laminare	selected	Millipore Corp., Massachusetts, USA
ARcare® 8192	plastic	tested	Adhesive Research, Limerick, Ireland
Sample pad			
designation	material	note	
CFSP 173000	cellulose	original	Millipore Corp., Massachusetts, USA
CFSP 223000	cellulose	tested	Millipore Corp., Massachusetts, USA
FUSION 5	glass and synthetic material	tested	Whatman GmbH, Dassel, Germany
Grade 121	glass and synthetic material	tested	Ahlstrom, Kotka, Finland
Grade 142	glass and synthetic material	tested	Ahlstrom, Kotka, Finland
Grade 1281	cotton	selected	Ahlstrom, Kotka, Finland
Conjugation pad			
designation	material	note	
GFCP 103000	glass fibers	original	Millipore Corp., Massachusetts, USA
CFDX 103000	glass fibers	tested	Millipore Corp., Massachusetts, USA
Grade 6613	polyester	tested	Ahlstrom, Kotka, Finland
Grade 6615	polyester	selected	Ahlstrom, Kotka, Finland
Grade 8950	glass fibers	tested	Ahlstrom, Kotka, Finland
Grade 8951	glass fibers	tested	Ahlstrom, Kotka, Finland
Absorption pad			
designation	material / thickness [mm]	note	
CFSP 173000	cellulose / 0.83	original	Millipore Corp., Massachusetts, USA
CFSP 223000	cellulose / 0.83	tested	Millipore Corp., Massachusetts, USA
Grade 222	cotton / 0.83	tested	Ahlstrom, Kotka, Finland
Grade 237	cotton / 0.42	tested	Ahlstrom, Kotka, Finland
Grade 238	cotton / 0.34	tested	Ahlstrom, Kotka, Finland
Grade 319	cotton / 0.48	tested	Ahlstrom, Kotka, Finland
Grade 320	cotton / 2.48	selected	Ahlstrom, Kotka, Finland
Grade 601	cotton / 0.19	tested	Ahlstrom, Kotka, Finland

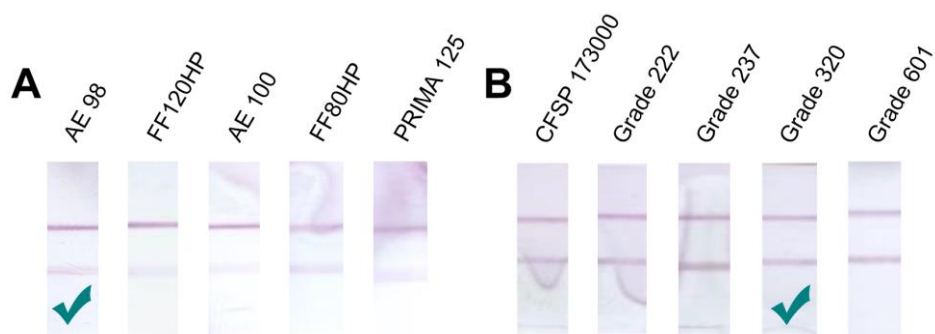


Figure S5. Some results of tested membranes (panel A) and absorbent pads (panel B) for indirect closed format.

Table S2. Summary of tested drying and reaction buffers

Drying buffers
0.2 mol/L borate buffer-0.1% BSA-3% trehalose-1% Tween 20
0.1 mol/L borate buffer-0.1% BSA-3% trehalose-1% Tween 20
0.2 mol/L borate buffer-0.1% BSA-1% Tween 20
0.2 mol/L borate buffer-1% BSA-3% trehalose-1% Tween 20
0.2 mol/L borate buffer-0.1% BSA-3% trehalose-0.5% Tween 20
0.1 mol/L borate buffer-1% BSA-3% trehalose-1% Tween 20
0.1 mol/L borate buffer-0.1% BSA-3% trehalose-0.5% Tween 20
Reaction buffers
0.1 mol/L borate buffer-1% BSA-1% PEG- 1%Tween 20
0.2 mol/L borate buffer-1% BSA-1% PEG- 1%Tween 20
0.1 mol/L borate buffer-1% PEG-1% Tween 20
0.1 mol/L borate buffer-1% BSA-1% Tween 20
0.1 mol/L borate buffer-1% BSA-1% PEG
0.1 mol/L borate buffer-0.5% BSA-1% PEG- 1%Tween 20
0.1 mol/L borate buffer-2% BSA-1% PEG- 1%Tween 20
0.1 mol/L borate buffer-1% BSA-0.5% PEG- 1%Tween 20
0.1 mol/L borate buffer-1% BSA-2% PEG- 1%Tween 20
0.1 mol/L borate buffer-1% BSA-1% PEG- 0.5%Tween 20
0.1 mol/L borate buffer-1% BSA-1% PEG- 2%Tween 20

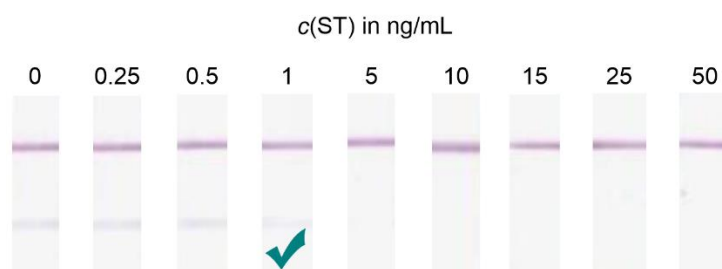


Figure S6. Calibration series for indirect closed format (3 μ L GAR-AuNPs were applied).

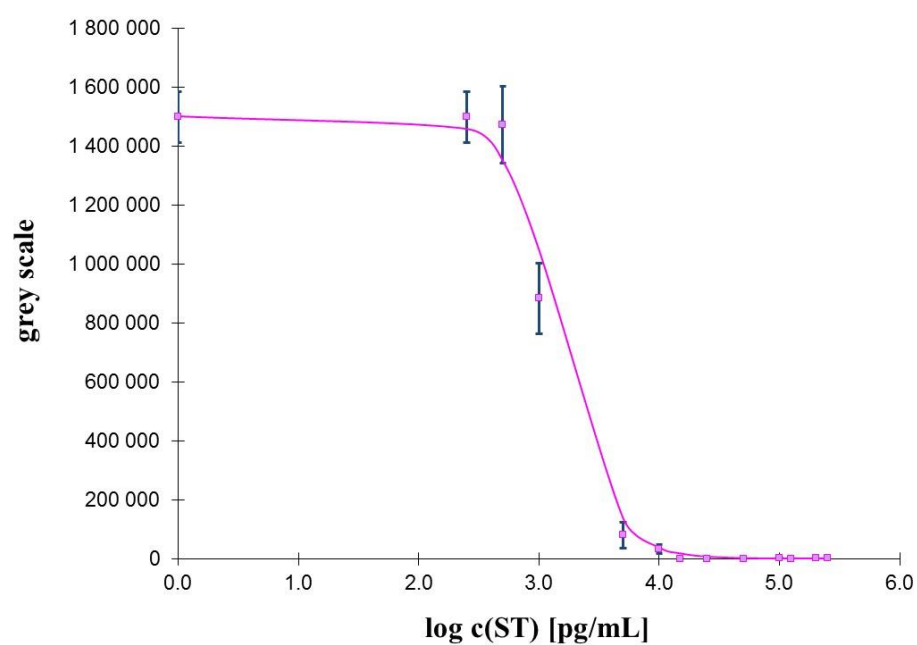


Figure S7. Calibration curve for indirect ICT format. The error bars correspond to the standard deviations of the data points (n = 3)

Table S3. Characteristics of ST standard curves for indirect and direct format (data from 3 analyses).

ICT format	Linear working range ^a (ng/mL)	Limit of detection $\pm$ SD ^b (ng/mL)	IC ₅₀ $\pm$ SD ^b (ng/mL)
direct	0.75 – 3.13	0.34 $\pm$ 0.09	1.4 $\pm$ 0.4
indirect	0.41 – 3.8	0.42 $\pm$ 0.12	1.6 $\pm$ 0.4

^a Expressed as the concentration range causing 20-80% inhibition of the maximal assay signal.

^b Standard deviation.

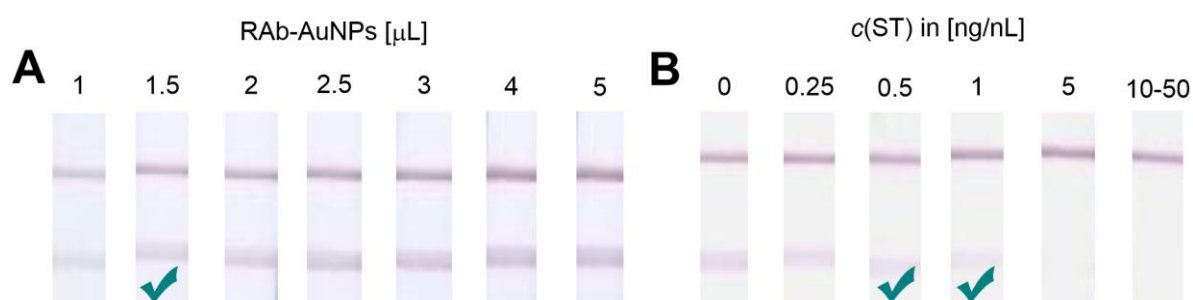


Figure S8. Selection of the applied amount of RAb-AuNPs on panel A and the calibration series for direct open format (1.5 µL RAb-AuNPs were applied) on panel B.

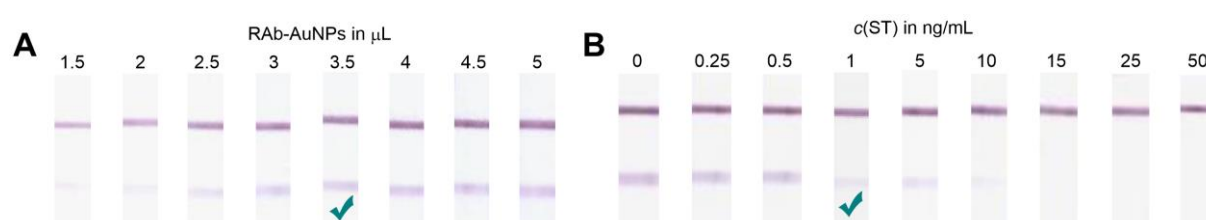


Figure S9. Tested amounts of applied RAb-AuNPs for closed format (panel A) and calibration series for direct closed format (3.5 µL of RAb-AuNPs was applied; panel B).

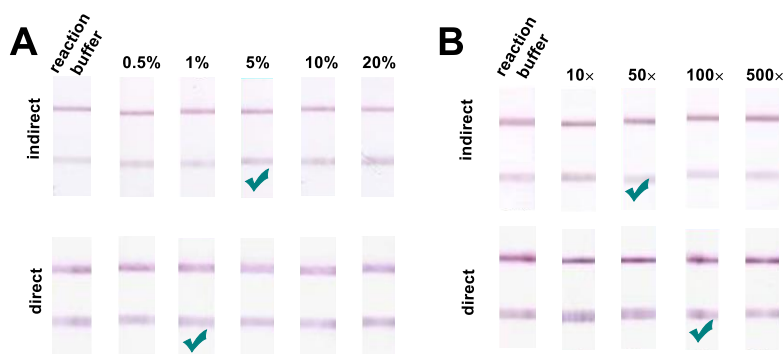


Figure S10. Testing of extraction solvent (panel A) and matrix effect (panel B) on the functionality of RAb 212 antibody.

Table S4. Matrices used for contamination with AAS

Food supplement			Manufacturer / supplier
Matrix No.	designation	content	-
1	100% whey protein	ultrafiltered whey protein	SCITEC NUTRITION, Orlando, FL, USA
2	Gain Tech	weight gainer	ALL STARS, Peißenberg, DE
3	Magnus	weight gainer	PENCO, Řevnice, CZ
4	Compress B.I.G.	instant gainer	NUTREND D.S., Olomouc, CZ
5	TRUE-MASS	mass gainer	Bio-Engineered Supplements & Nutrition, Boca Raton, FL, USA
6	NITRIX	Vaso-muscular volumizer	Bio-Engineered Supplements & Nutrition, Boca Raton, FL, USA
7	AMIX EGG AMINO 6000	fat-free amino formula	Large Life Ltd., Manchester, UK
8	METHOXY-7-TEST	anabolic support complex	PhD Nutrition Ltd., Hull, UK
9	BCAA Complex 2200	branched-chain amino acids	Dymatize Enterprises Inc., Dallas, TX, USA
10	BCAA X	BCAA muscle preservation system	SCITEC NUTRITION, Orlando, FL, USA

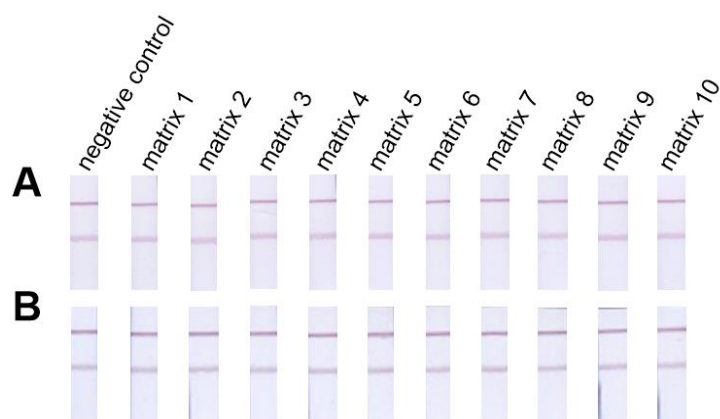


Figure S11. Tested effect of selected matrices in indirect (panel A) and direct format (panel B) opened ICT format. The designation of the matrices is given above in Table S3.

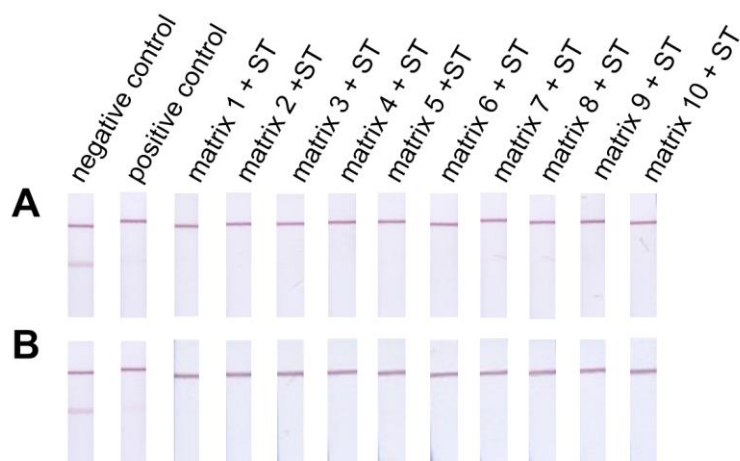


Figure S12. Tested selected matrices with the addition of ST with 250× dilution; indirect (panel A) and direct (panel B) opened ICT format.

References

1. Huml, L.; Havlová, D.; Longin, O.; Staňková, E.; Holubová, B.; Kuchař, M.; Prokudina, E.; Rottnerová, Z.; Zimmermann, T.; Drašar, P.; Lapčík, O.; Jurášek, M., Stanazolol derived ELISA as a sensitive forensic tool for the detection of multiple 17 α -methylated anabolics. *Steroids* **2020**, *155*, 108550.