



- (51) **International Patent Classification:**
G01N 33/58 (2006.01) C12Q 1/68 (2006.01)
- (21) **International Application Number:**
PCT/CZ2015/000084
- (22) **International Filing Date:**
4 August 2015 (04.08.2015)
- (25) **Filing Language:** Czech
- (26) **Publication Language:** English
- (30) **Priority Data:**
PV 2014-527 5 August 2014 (05.08.2014) CZ
- (71) **Applicant:** USTAV ORGANICKE CHEMIE A BIO-CHEMIE AKADEMIE VED CR, V.V.I [CZ/CZ]; Flemingovo namesti 542/2, 166 10 Praha 6 (CZ).
- (72) **Inventors:** NAVRATIL, Vaclav; Na Vysoke I 16, 150 00 Praha 5 (CZ). SACHA, Pavel; K mostku 5, 163 00 Praha 6 (CZ). SCHIMER, Jiri; Wichterlova 10, 182 00 Praha 8 (CZ). KONVALINKA, Jan; Nova Sarka 39, 161 00 Prague 6 (CZ). MAJER, Pavel; Sturova 30, 142 00 Praha 4 (CZ).
- (74) **Agent:** HARTVICOVA, Katerina; INVENTIA s.r.o., Na Belidle 3, 150 00 Praha 5 (CZ).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

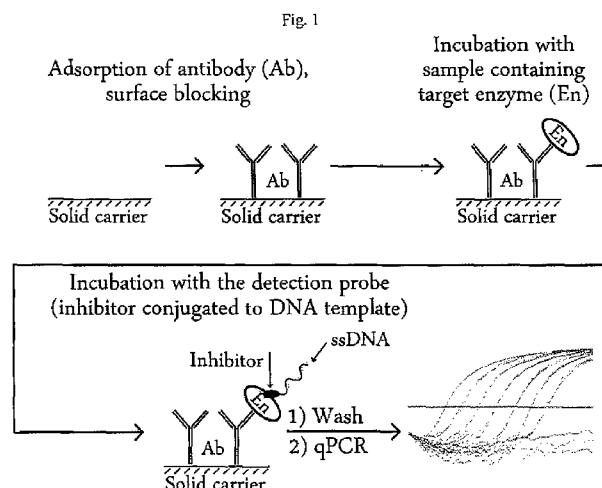
(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

[Continued on next page]

(54) **Title:** METHOD OF DETECTION OF ANALYTE ACTIVE FORMS AND DETERMINATION OF THE ABILITY OF SUBSTANCES TO BIND INTO ANALYTE ACTIVE SITES



(57) **Abstract:** The invention provides a method for detection of active form of analytes in a sample and/or for determination of ability of tested substances to bind to the active site of these analytes, comprising the following steps: a) analyte or group of analytes from the sample is immobilized on the surface of a solid carrier either by non-specific non-covalent adsorption or by covalent binding of surface functional groups of the analyte and corresponding functional groups of the solid carrier, or preferably via a binding molecule which is bound to the surface of the solid carrier before immobilization of the analyte or group of analytes and is capable of selectively binding the analyte or group of analytes contained in the sample during incubation of the solid carrier with the sample; b) analyte or group of analytes is incubated with a detection probe which binds selectively to the analyte or group of analytes via a compound for selective binding to the analyte active site; whereas the probe consists of a low molecular compound for selective binding to the analyte active site; an oligonucleotide tag, optionally with a covalently attached fluorophore, biotin or a chemical group, and a chemical linker covalently linking the compound for selective binding to the analyte active site and the oligonucleotide tag; c) then the solid carrier is washed to remove unbound detection probe; and subsequently, the amount of bound detection probe is determined, whereas this amount is directly

[Continued on next page]



— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
31 March 2016

proportional to the amount of the analyte or group of analytes in the sample. The described method has broad application in medicine. Given the exceptional sensitivity of only a few dozen molecules, it provides the ability to determine the protein markers in blood in a concentration yet undetectable.