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Instructions for Use

Adalimumab (Humira®) ELISA

SHIKARI® Q-ADA

Enzyme immunoassay for the quantitative determination of adalimumab (Humira®) in serum and plasma

REF TR-ADAv1  12 x 8    2-8°C

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Matriks Biotek® Laboratories
www.matriksbiotek.com

Contents	Page
Intended Use	3
Summary and Explanation	3
Test Principle	5
Warnings and Precautions	6
Storage and Stability of the Kit	7
Specimen Collection and Storage	7
Materials Supplied	8
Materials Required but not Supplied	8
Procedure Notes	9
Pre-Test Setup Instruction	9
Test Procedure	10
Quality Control	11
Calculation & Interpretation of Results	11
Assay Characteristics	13
Automation	13
References	14
Semi Log Graph Paper	17
Semi Log Graph Paper	18

	SHIKARI Q-ADA
	Free adalimumab (Humira®) quantitative analyses
Required Volume (µl)	20
Total Time (min)	70
Sample	Serum, plasma
Sample Number	96
Detection Limit (ng/mL)	10
Spike Recovery (%)	Between 85-115
Shelf Life (year)	1

Intended Use

Enzyme immunoassay for the quantitative determination of free adalimumab (Humira®) in serum and plasma. Matriks Biotek® adalimumab ELISA has been especially developed for the quantitative analysis of free adalimumab in serum and plasma samples.

Summary And Explanation

Adalimumab (Humira®) is a recombinant human IgG1 monoclonal antibody specific for human tumor necrosis factor alpha (TNF- α). Adalimumab (Humira®) was created using phage display technology resulting in an antibody with human derived heavy and light chain variable regions and human IgG1:k constant regions. Adalimumab (Humira®) is produced by recombinant DNA technology in a mammalian cell expression system and is purified by a process that includes specific viral inactivation and removal steps. It consists of 1330 amino acids and has a molecular weight of approximately 148 kilodaltons.

Adalimumab (Humira®) binds specifically to (TNF- α) and blocks its interaction with the p55 and p75 cell surface TNF receptors. Adalimumab (Humira®) also lyses surface TNF expressing cells in vitro in the presence of complement. Adalimumab (Humira®) does not bind or inactivate lymphotoxin (TNF- β). TNF is a naturally occurring cytokine that is involved in normal inflammatory and immune responses. Elevated levels of TNF are found in the synovial fluid of rheumatoid arthritis, including juvenile idiopathic arthritis, psoriatic arthritis, and ankylosing spondylitis patients and play an important role in both the pathologic inflammation and the joint destruction that are hallmarks of these diseases. Increased levels of TNF are also found in psoriasis (Ps) plaques.

After treatment with adalimumab (Humira®), a decrease in levels of acute phase reactants of inflammation (C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR]) and serum cytokines (IL-6) was observed compared to baseline in patients with rheumatoid arthritis. A decrease in CRP levels was also observed in patients with Crohn's disease. Serum levels of matrix metalloproteinases (MMP-1 and MMP-3) that produce tissue remodeling responsible for cartilage destruction were also decreased after adalimumab (Humira®) administration.

According to the prospectus; the maximum serum concentration (C_{max}) and the time to reach the maximum concentration (T_{max}) were $4.7 \pm 1.6 \mu\text{g/mL}$ and 131 ± 56 hours respectively, following a single 40 mg subcutaneous administration of

adalimumab (Humira®) to healthy adult subjects. The average absolute bioavailability of adalimumab (Humira®) estimated from three studies following a single 40 mg subcutaneous dose was 64%. The pharmacokinetics of adalimumab were linear over the dose range of 0.5 to 10.0 mg/kg following a single intravenous dose. The single dose pharmacokinetics of adalimumab in rheumatoid arthritis (RA) patients were determined in several studies with intravenous doses ranging from 0.25 to 10 mg/kg. The distribution volume (V_{ss}) ranged from 4.7 to 6.0 L. The systemic clearance of adalimumab is approximately 12 mL/hr. The mean terminal half-life was approximately 2 weeks, ranging from 10 to 20 days across studies. Adalimumab concentrations in the synovial fluid from five rheumatoid arthritis patients ranged from 31 to 96% of those in serum. In RA patients receiving 40 mg adalimumab (Humira®) every other week, adalimumab mean steady-state trough concentrations of approximately 5 µg/mL and 8 to 9 µg/mL, were observed without and with 3 methotrexate (MTX), respectively. In patients with RA, MTX reduced adalimumab apparent clearance after single and multiple dosing by 29% and 44% respectively. Mean serum adalimumab trough levels at steady state increased approximately proportionally with dose following 20, 40, and 80 mg every other week and every week subcutaneous dosing. In long-term studies with dosing more than two years, there was no evidence of changes in clearance over time. Adalimumab mean steady-state trough concentrations were slightly higher in psoriatic arthritis patients treated with 40 mg adalimumab (Humira®) every other week (6 to 10 µg/mL and 8.5 to 12 µg/mL, without and with MTX, respectively) compared to the concentrations in RA patients treated with the same dose. The pharmacokinetics of adalimumab in patients with ankylosing spondylitis were similar to those in patients with RA. In patients with Crohn's disease, the loading dose of 160 mg adalimumab (Humira®) on week 0 followed by 80 mg adalimumab (Humira®) on week 2 achieves mean serum adalimumab trough levels of approximately 12 µg/mL at week 2 and week 4. Mean steady-state trough levels of approximately 7 µg/mL were observed at week 24 and week 56 in Crohn's disease patients after receiving a maintenance dose of 40 mg adalimumab (Humira®) every other week. In patients with plaque psoriasis, the mean steady-state trough concentration was approximately 5 to 6 µg/mL during adalimumab 40 mg every other week monotherapy treatment.

Population pharmacokinetic analyses in patients with RA revealed that there was a trend toward higher apparent clearance of adalimumab in the presence of antiadalimumab antibodies, and lower clearance with increasing age in patients aged 40 to >75 years. Minor increases in apparent clearance were also predicted in RA patients receiving doses lower than the recommended dose and in RA patients with high rheumatoid factor or CRP concentrations. These increases are reported to be not likely to be clinically important.

In subjects with juvenile idiopathic arthritis (4 to 17 years of age), the mean steady-state trough serum adalimumab concentrations for subjects weighing <30 kg receiving 20 mg adalimumab (Humira®) subcutaneously every other week as monotherapy or with concomitant methotrexate were 6.8 µg/mL and 10.9 µg/mL, respectively. The mean steady-state trough serum adalimumab concentrations for subjects weighing ≥30 kg receiving 40 mg adalimumab (Humira®) subcutaneously every other week as monotherapy or with concomitant methotrexate were 6.6 µg/mL and 8.1 µg/mL, respectively.

It was reported that doses up to 10 mg/kg have been administered to patients in clinical trials without evidence of dose limiting toxicities. However, in case of overdose, it is recommended that the patient be monitored for any signs or symptoms of adverse reactions or effects and appropriate symptomatic treatment instituted immediately.

However, no pharmacokinetic data are available in patients with hepatic or renal impairment. Serum concentration of adalimumab (Humira®) might be related to predict some clinical outcome during maintenance therapy. It was also possible that the surveillance of circulating adalimumab (Humira®) concentration during maintenance therapy represents a direct and/or indirect factor for some other side effects. In this context, identification of biomarkers for (non-)response and risk factors for adverse drug reactions that might be related to serum concentrations and maintaining the effective minimum concentration of adalimumab (Humira®) in order to potentially avoid some side effects with a reliable method might be beneficial.

Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. Standards and diluted samples (serum or plasma) are incubated in the microtitre plate coated with the reactant for adalimumab (Humira®). After incubation, the wells are washed. A horse radish peroxidase (HRP) conjugated probe is added and binds to adalimumab (Humira®) captured by the reactant on the surface of the wells. Following incubation, wells are washed and the bound enzymatic activity is detected by addition of chromogen-substrate. The colour developed is proportional to the amount of adalimumab in the sample or standard. Results of samples can be determined directly using the standard curve.

Warnings And Precautions

1. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.
3. In case of severe damage of the kit package please contact Matriks Biotek® or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents of this kit containing human serum or plasma (i.e. standards) have been tested and were found negative for HIV I/II, HBsAg and HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.
10. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.

Storage and Stability

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The strips of microtiter plate is stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2–8°C.

Specimen Collection and Storage

Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light Avoid repeated freeze-thaw cycles
Stability:	2 d	6 mon	

*. Adalimumab (Humira®) infusion camouflages/masks the presence of antibody to Adalimumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of Adalimumab. The Matriks Biotek® Laboratories suggests to obtain blood sample just before the infusion of Adalimumab (Humira®) or at least 2 weeks after the infusion of Adalimumab (Humira®).

Materials Supplied

1 x 12 x 8	MTP	Microtiter Plate Break apart strips. Microtiter plate with 12 rows each of 8 wells coated with reactant.
5 x 0.3 mL	STND A-E	Adalimumab Standards A-E 1000; 300; 100; 30; 0 nanogram/mL Ready to use. Used for construction of the standard curve. Contains adalimumab (Humira®), human serum, stabilizer and <0.1% NaN3.
1 x 50 mL	ASSAY BUF	Assay Buffer Blue colored. Ready to use. Contains proteins and <0.1% NaN3.
1 x 12 mL	HRP CONJ	Horse radish peroxidase-Conjugated Probe Red colored. Ready to use. Contains HRP-probe, stabilizer and <0.1% NaN3.
1 x 12 mL	TMB SUBS	TMB Substrate Solution Ready to use. Contains TMB
1 x 12 mL	TMB STOP	TMB Stop Solution Ready to use. 1N HCl.
1 x 50 mL	WASHBUF CONC	Wash Buffer, Concentrate (20x) Contains Buffer with Tween 20.
2 x 1	FOIL	Adhesive Foil For covering of Microtiter Plate during incubation.
2x 1	SLGP	Semi-Log Graph Paper For constructing standard curve and calculation of results.

Materials Required but not Supplied

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV).
2. Calibrated measures.
3. Tubes (1 mL) for sample dilution.
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of reading absorbance at 450/650 nm.
6. Bidistilled or deionised water, paper towels, pipette tips and timer.

Procedure Notes

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

Pre-Test Setup Instructions

1. Preparation of Components

Dilute/ disolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
10 mL	Wash Buffer*	Up to 200 mL	bidist. Water	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	4 w

*. Prepare Wash Buffer before starting assay procedure.

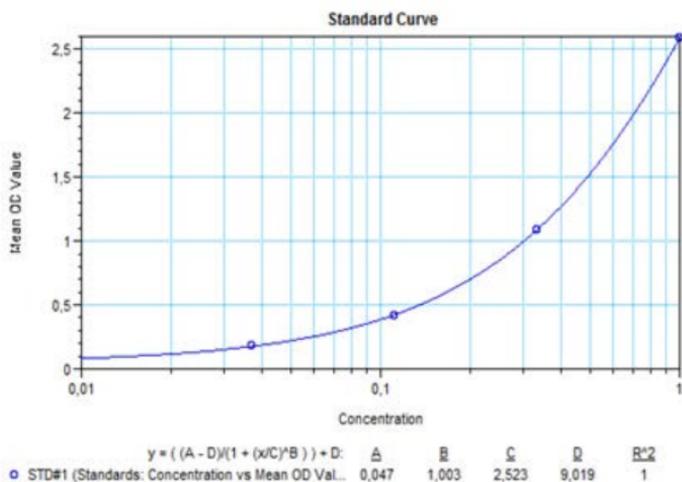
- To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (10x). Any sample reading greater than the highest standard should be further diluted appropriately with assay buffer and retested. Therefore, if the pre-diluted samples have been further diluted, the concentration determined from the standard curve must be multiplied by the further dilution factor.

E.g.; If the pre-diluted sample further diluted in a ratio of 1:10 then results should be multiplied by 100.

- Automated method: computer programs also generally use a good fit.

Typical Calibration Curve

(Example. Do not use for calculation!)



Standard	Concentration (ng/mL)	Mean OD450/650 nm
A	1000	2,248
B	300	1,287
C	100	0,493
D	30	0,204
E	0	0,055

Assay Characteristics

- 1. Specificity:** There is no cross reaction with native serum immunoglobulins. However, cross reaction is observed with the serum/plasma samples of patients under the treatment of other therapeutic anti-TNF antibodies such as infliximab (Remicade®) and/or etanercept (Enbrel®).
- 2. Sensitivity:** The lowest detectable level that can be distinguished from the zero standard is less than 10 ng/mL.
- 3. Precision of Kit:**
 - Intra-assay CV: <5% for adalimumab range 30-1000 ng/mL.
 - Inter-assay CV: <20% for adalimumab range 30-1000 ng/mL.
- 4. Recovery:** Recovery rate was found to be equal and higher than 95% with normal human serum samples with known concentrations.

Automation

Experiments have shown that the adalimumab ELISA is also suitable to run on an automated ELISA processor.

References

1. Kempeni J. Preliminary results of early clinical trials with the fully human anti-TNFalpha monoclonal antibody D2E7., *Ann Rheum Dis.* 1999 Nov;58 Suppl 1:170-2.
2. Kempeni J. Update on D2E7: a fully human anti-tumour necrosis factor alpha monoclonal antibody., *Ann Rheum Dis.* 2000 Nov;59 Suppl 1:i44-5.
3. Taylor PC., Anti-tumor necrosis factor therapies. *Curr Opin Rheumatol.* 2001 May;13(3):164-9. Review.
4. Barrera P, Joosten LA, den Broeder AA, van de Putte LB, van Riel PL, van den Berg WB., Effects of treatment with a fully human anti-tumour necrosis factor alpha monoclonal antibody on the local and systemic homeostasis of interleukin 1 and TNFalpha in patients with rheumatoid arthritis. *Ann Rheum Dis.* 2001 Jul;60(7):660-9.
5. Lorenz HM., Technology evaluation: adalimumab, Abbott laboratories. *Curr Opin Mol Ther.* 2002 Apr;4(2):185-90.
6. den Broeder AA, Joosten LA, Saxne T, Heinegård D, Fenner H, Miltenburg AM, Frasa WL, van Tits LJ, Buurman WA, van Riel PL, van de Putte LB, Barrera P., Long term anti-tumour necrosis factor alpha monotherapy in rheumatoid arthritis: effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. *Ann Rheum Dis.* 2002 Apr;61(4):311-8.
7. Den Broeder AA, Creemers MC, van Gestel AM, van Riel PL., Dose titration using the Disease Activity Score (DAS28) in rheumatoid arthritis patients treated with anti-TNF-alpha. *Rheumatology (Oxford).* 2002 Jun;41(6):638-42.
8. den Broeder A, van de Putte L, Rau R, Schattenkirchner M, Van Riel P, Sander O, Binder C, Fenner H, Bankmann Y, Velagapudi R, Kempeni J, Kupper H., A single dose, placebo controlled study of the fully human anti-tumor necrosis factor-alpha antibody adalimumab (D2E7) in patients with rheumatoid arthritis. *J Rheumatol.* 2002 Nov;29(11):2288-98.
9. Weinblatt ME, Keystone EC, Furst DE, Moreland LW, Weisman MH, Birbara CA, Teoh LA, Fischkoff SA, Chartash EK., adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum.* 2003 Jan;48(1):35-45. Erratum in: *Arthritis Rheum.* 2003 Mar;48(3):855.
10. Taylor PC., Anti-TNFalpha therapy for rheumatoid arthritis: an update. *Intern Med.* 2003 Jan;42(1):15-20. Review.
11. Sandborn WJ., Strategies for targeting tumour necrosis factor in IBD. *Best Pract Res Clin Gastroenterol.* 2003 Feb;17(1):105-17. Review.
12. van de Putte LB, Rau R, Breedveld FC, Kalden JR, Malaise MG, van Riel PL, Schattenkirchner M, Emery P, Burmester GR, Zeidler H, Moutsopoulos HM, Beck K, Kupper H., Efficacy and safety of the fully human anti-tumour necrosis factor alpha monoclonal antibody adalimumab (D2E7) in DMARD refractory patients with rheumatoid arthritis: a 12 week, phase II study. *Ann Rheum Dis.* 2003 Dec;62(12):1168-77.

13. Papadakis KA, Shaye OA, Vasiliauskas EA, Ippoliti A, Dubinsky MC, Birt J, Paavola J, Lee SK, Price J, Targan SR, Abreu MT., Safety and efficacy of adalimumab (D2E7) in Crohn's disease patients with an attenuated response to infliximab. *Am J Gastroenterol.* 2005 Jan;100(1):75-9.
14. den Broeder AA, Joosten LA, Saxne T, Heinegård D, Fenner H, Miltenburg AM, Frasa WL, van Tits LJ, Buurman WA, van Riel PL, van de Putte LB, Barrera P., Long term antitumour necrosis factor alpha monotherapy in rheumatoid arthritis: effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. *Ann Rheum Dis.* 2002 Apr;61(4):311-8.
15. Ghate JV, Alspaugh CD., adalimumab in the management of palmoplantar psoriasis. *J Drugs Dermatol.* 2009 Dec;8(12):1136-9.
16. Jürgens M, Brand S, Filik L, Hübener C, Hasbargen U, Beigel F, Tillack C, Göke B, Ochsenkühn T, Seiderer J., Safety of adalimumab in Crohn's disease during pregnancy: Case report and review of the literature. *Inflamm Bowel Dis.* 2009 Dec 21. [Epub ahead of print] No abstract available.
17. Murphy G, Schmidt-Martin D, Hynes BG, Harney S., Systemic listeriosis with adalimumab therapy. *J Clin Rheumatol.* 2009 Oct;15(7):369-70.
18. Bret J, Hernandez J, Aquilina C, Zabraniecki L, Fournie B., Kaposi's disease in a patient on adalimumab for rheumatoid arthritis. *Joint Bone Spine.* 2009 Dec;76(6):721-2.
19. Martorell-Calatayud A, Balmer N, Cardenas Cardona LF, Teague D., Interstitial Granulomatous Drug Reaction to adalimumab. *Am J Dermatopathol.* 2009 Nov 13.
20. Mease PJ, Signorovitch J, Yu AP, Wu EQ, Gupta SR, Bao Y, Mulani PM., Impact of adalimumab on Symptoms of Psoriatic Arthritis in Patients with Moderate to Severe Psoriasis: A Pooled Analysis of Randomized Clinical Trials. *Dermatology.* 2009 Nov 19.
21. Turner D, Picot J, Cooper K, Loveman E., adalimumab for the treatment of psoriasis. *Health Technol Assess.* 2009 Sep;13 Suppl 2:49-54.
22. Ghate JV, Alspaugh CD., adalimumab in the management of palmoplantar psoriasis. *Dermatol Online J.* 2009 Jul 15;15(7):15.
23. Wetter DA, Davis MD., Lupus-like syndrome attributable to anti-tumor necrosis factor alpha therapy in 14 patients during an 8-year period at Mayo Clinic. *Mayo Clin Proc.* 2009 Nov;84(11):979-84.
24. Borrás-Blasco J, Navarro-Ruiz A, Borrás C, Casterá E., Adverse cutaneous reactions induced by TNF-alpha antagonist therapy. *South Med J.* 2009 Nov;102(11):1133-40. Review.
25. Irla N, Yawalkar N., Marked improvement in nail psoriasis during treatment with adalimumab. *Dermatology.* 2009;219(4):353-6. Epub 2009 Oct 23.
26. Patel SR., Systemic sarcoidosis with bone marrow involvement responding to therapy with adalimumab: a case report. *J Med Case Reports.* 2009 Jul 29;3:8573.
27. de Vries MK, Brouwer E, van der Horst-Bruinsma IE, Spoorenberg A, van Denderen JC, Jamnitski A, Nurmohamed MT, Dijkmans BA, Aarden LA, Wolbink GJ., Decreased clinical response to adalimumab in ankylosing spondylitis is associated with antibody formation. *Ann Rheum Dis.* 2009 Nov;68(11):1787-8.

28. Murdaca G, Colombo BM, Puppo F., Anti-TNF-alpha inhibitors: a new therapeutic approach for inflammatory immune-mediated diseases: an update upon efficacy and adverse events. *Int J Immunopathol Pharmacol.* 2009 Jul-Sep;22(3):557-65. Review.
29. van den Bosch F, Manger B, Goupille P, McHugh N, Rødevand E, Holck P, van Vollenhoven RF, Leirisalo-Repo M, Fitzgerald O, Kron M, Frank M, Kary S, Kupper H., Effectiveness of adalimumab in treating patients with active psoriatic arthritis (PsA) and predictors of good clinical responses for arthritis, skin, and nail lesions. *Ann Rheum Dis.* 2009 Oct 7. [Epub ahead of print]
30. Varani K, Massara A, Vincenzi F, Tosi A, Padovan M, Trotta F, Borea PA., Normalization of A2A and A3 adenosine receptor up-regulation in rheumatoid arthritis patients by treatment with anti-tumor necrosis factor alpha but not methotrexate. *Arthritis Rheum.* 2009 Oct;60(10):2880-91.
31. Salama M, Lawrance IC., Stevens-Johnson syndrome complicating adalimumab therapy in Crohn's disease. *World J Gastroenterol.* 2009 Sep 21;15(35):4449-52.
32. Cassinotti A, Ardizzone S, Porro GB., adalimumab for the treatment of Crohn's disease. *Biologics.* 2008 Dec;2(4):763-77.
33. Yildiz N, Alkan H, Ardic F, Topuz O., Successful treatment with adalimumab in a patient with coexisting Behçet's disease and ankylosing spondylitis. *Rheumatol Int.* 2009 Aug 25. [Epub ahead of print]
34. Tong D, Eather S, Manolios N., Psoriatic arthritis and chronic lymphoedema: treatment efficacy by adalimumab. *Clin Rheumatol.* 2009 Nov;28(11):1349-50. Epub 2009 Aug 20.
35. Bradford RD, Pettit AC, Wright PW, Mulligan MJ, Moreland LW, McLain DA, Gnann JW, Bloch KC., Herpes simplex encephalitis during treatment with tumor necrosis factor-alpha inhibitors. *Clin Infect Dis.* 2009 Sep 15;49(6):924-7.
36. Barreiro-de Acosta M, Lorenzo A, Dominguez-Muñoz JE., adalimumab in ulcerative colitis: two cases of mucosal healing and clinical response at two years. *World J Gastroenterol.* 2009 Aug 14;15(30):3814-6.
37. Karmiris K, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D, Claes K, Coopman T, Van Schuerbeek N, Van Assche G, Vermeire S, Rutgeerts P., Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology.* 2009 Nov;137(5):1628-40. Epub 2009 Aug 5.

