ADVANCED BIOLOGICALS TESTING
Therapeutic drug monitoring is vitally important to optimize therapy for all patients treated with biologicals. Routine measurement of serum drug levels brings both under and overtreatment to light. Smart testing biologicals enables personalized medicine: cost-effective fine-tuning of drug dosage or an early switch to other therapeutics. For over a decade, Sanquin has offered service testing for the pharmacokinetics and immunogenicity of an expanding array of biologicals, using assays developed in-house. Sanquin is the frontrunner in the development of new tests for the most recently introduced biologicals.
Biologicals have had a profound impact on various medical fields, primarily rheumatology and oncology, but also cardiology, dermatology, gastroenterology, neurology, and other areas. In most of these disciplines, biologicals have added major opportunities for the treatment of disease, making this type of medication the fastest growing class of therapeutics today. Monoclonal antibodies targeting tumor necrosis factor (TNF) were among the first biologicals to appear on the market. They have proved to be powerful drugs applied for such inflammatory diseases as rheumatoid arthritis, ankylosing spondylitis, psoriasis and Crohn’s disease. However, biologicals belong to the most expensive drugs available. It is therefore vital that they are applied in a sensible, cost-effective manner.

The growing field of biologicals

All patients may benefit from routine measurement of serum trough levels of biologicals (therapeutic drug monitoring, or TDM). TDM aims to improve patient care by adjusting the drug dosage at the individual patient level. TDM identifies patients who are undertreated as well as those who are overtreated. Low serum drug levels are associated with the lack or loss of clinical response. On the other hand, in patients with levels higher than necessary, decreasing drug dosing results in higher cost effectiveness. Low serum drug levels are frequently caused by the immunogenicity of the biological. Therefore, measurement of antidrug antibodies (ADA) in sera with low serum drug levels aids in further clinical decision making, because the presence or absence of ADA to the first biological has implications for the choice of an alternative biological. Patients not responding to therapy, with low drug levels and ADA formation, may switch to a biological with a similar target. In contrast, non-responders with adequate drug levels who do not develop ADA may benefit from biologicals with another mode of action. Thus, TDM and ADA testing may support clinical decision making and enable therapy optimization for individual patients. The personalized medicine approach benefits the patient and reduces cost by preventing overtreatment or treatment with an ineffective drug.

Personalized medicine and cost effectiveness
**Decision-making example.** When drug levels are decreased, the presence/absence of antidrug antibodies guides the next treatment step. In addition, reducing drug dosage in well-responding patients with relatively high drug levels cuts down cost.

Note: This is only a general scheme. Kinetics, dosing schedules and effective levels differ between biologics and diseases. If you have further questions, please contact Sanquin (biologicaals@sanquin.nl) to discuss your results.
To apply personalized medicine, target drug levels should be known. For that purpose researchers of Sanquin together with Reade, Centre for Rehabilitation and Rheumatology in Amsterdam, determined the concentration-effect curve of adalimumab. In a cohort of 221 adalimumab-treated rheumatoid arthritis (RA) patients, drug concentration was related to drug effectiveness. Adalimumab trough levels reached a maximal effect on RA disease activity at 5 to 8 μg/ml. Levels higher than 8 μg/ml had no additional benefit. In patients with higher concentrations the dose interval may be lowered without losing clinical efficacy, thereby saving cost. More research is needed to elucidate optimal drug levels for other diseases and biologics.

The concentration-effect curve of adalimumab in RA. Clinical efficacy, expressed as delta DAS28 score (differences in DAS28 scores between baseline and week 28) improved with increasing adalimumab concentration, and reached a maximum (mean DAS28 improvement of 2) with levels of 5 to 8 μg/ml. (Pouw and Krieckaert, 2013).
Biologicals may elicit an unwanted antibody response, also known as immunogenicity, resulting in a decreased level of active drug and loss of efficacy of treatment. Biologicals may be recognized by the human immune system as ‘non self’ and induce an immune response. Therapeutic antibodies, even those that are fully human, carry unique stretches of amino acids, which form the antigen-binding site and determine their specificity. These sequences are frequently seen as foreign by the immune system of the patient and induce the formation of antidrug antibodies (ADA). ADA can form a complex with the drug, thereby inhibiting drug activity, which can result in treatment failure. ADA are also associated with adverse events, of which infusion reactions are most common. Beside the nature of the biological, antibody responses may also be influenced by patient-related factors such as genetic background, underlying disease, immunomodulating therapy, and dosing schedule.

**Typical profile of an infliximab treated patient with an anti-drug antibody response.** Trough serum levels of infliximab, anti-drug antibodies and the Disease Activity Score in 28 joints (DAS28) in a rheumatoid arthritis patient with a typical immune response against infliximab. After initial decrease of disease activity, the patient had a relapse of disease activity that coincided with a decrease in serum trough levels of infliximab and an increase in the anti-infliximab titre. AU= arbitrary units (Wolbink, 2006).
Drug level and immunogenicity testing

With over 30 years of experience in assay development, longstanding expertise in the field of autoimmune diseases and close interaction with the clinic, Sanquin offers service testing using a large number of assays to monitor biological therapeutics and their immunogenicity in clinical practice. Levels of therapeutics are assessed using validated ELISAs, while ADA are quantified using validated radioimmunoassays (antigen binding tests). To support reliable drug monitoring and increase precision and robustness, Sanquin has automated its drug level assays using state-of-the-art equipment, and optimized its reference preparations for enhanced accuracy. Our level tests are drug-specific and designed to detect only the active drug. Validated assays include (but are not limited to) those for infliximab, adalimumab, etanercept, certolizumab pegol and rituximab. In addition, Sanquin Reagents offers a range of ELISA kits to test levels and immunogenicity of biologicals in your own laboratory.

Development and co-development of level and immunogenicity tests

Sanquin standard assay formats allow for quick development of new tests. For the current status of our assays, please consult our website. Feel free to contact Sanquin to evaluate possibilities for co-development and see how we can best apply our knowledge and expertise for the benefit of your hospital, research group, or company. We look forward to discussing your (pre)-clinical program in detail.

Examples of commonly used biologicals

<table>
<thead>
<tr>
<th>Generic name</th>
<th>Brand name</th>
<th>Target</th>
<th>Disease</th>
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<tbody>
<tr>
<td>adalimumab</td>
<td>Humira®</td>
<td>TNF</td>
<td>• Inflammatory diseases, such as RA, ankylosing spondylitis and psoriasis</td>
</tr>
<tr>
<td>infliximab</td>
<td>Remicade®</td>
<td>TNF</td>
<td>• Inflammatory bowel disease</td>
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<tr>
<td>etanercept</td>
<td>Enbrel®</td>
<td>TNF</td>
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<td>certolizumab pegol</td>
<td>Cimzia®</td>
<td>TNF</td>
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<td>golimumab</td>
<td>Simponi®</td>
<td>TNF</td>
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<tr>
<td>abatacept</td>
<td>Orenchia®</td>
<td>CD80/CD86</td>
<td>Rheumatoid arthritis</td>
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<tr>
<td>tocilizumab</td>
<td>RoActemra®</td>
<td>IL-6 receptor</td>
<td>Rheumatoid arthritis</td>
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<td>natalizumab</td>
<td>Tysabri®</td>
<td>alpha-4 integrin</td>
<td>Multiple sclerosis</td>
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<tr>
<td>trastuzumab</td>
<td>Herceptin®</td>
<td>Her-2 receptor</td>
<td>Breast cancer</td>
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<tr>
<td>rituximab</td>
<td>MabThera®</td>
<td>B-cells</td>
<td>• Lymphomas</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Inflammatory diseases</td>
</tr>
<tr>
<td>ustekinumab</td>
<td>Stelara®</td>
<td>IL-12/IL-23</td>
<td>Psoriasis</td>
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Sanquin Blood Supply is responsible for safe and efficient blood supply in the Netherlands on a not-for-profit basis. Sanquin also develops and produces pharmaceutical products, conducts high-quality scientific research, and develops and performs a multitude of diagnostic services. Continuous research and innovation lead to new and improved products and services. Quality and development therefore go hand in hand.

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