Comment on Dana L. Baker et al. CMRO 2014; 1-10: printed May 2014, Vol. 30; No.5: 913-922) „Evaluation of two commercial omalizumab/free IgE immunoassays: implications of use during therapy“.

Dear Editor,

We have read your article (Dana L. Baker et al. CMRO 2014; 1-10: printed May 2014, Vol. 30; No.5: 913-922) „Evaluation of two commercial omalizumab/free IgE immunoassays: implications of use during therapy“ and we would like to comment on it.

The article describes the in-house testing (at Genentech) of two commercial kits for monitoring free IgE and omalizumab in the case of antibody therapy with Xolair.

You confirm that in cases in which treatment with the anti-IgE antibody omalizumab (Novartis trade name: Xolair) has been unsuccessful, the need for therapy monitoring has arisen.
However, you advise against therapy monitoring (free IgE and omalizumab) and refer to the official omalizumab-dosing table, although said dosing table is not up to date – at least for Europe.

For measuring free IgE with omalizumab therapy, you compared the BioTeZ recovery ELISA assay and the ViraCor-IBT free IgE assay with Genentech in-house free IgE ELISA assay and the omalizumab assay. Only the BioTeZ recovery ELISA assay is commercially available; other assays are not available for individual measurements.

The “in vivo samples” used in the article are obviously pooled patient sera and not native individual samples. In your investigation of these samples, you show that the functional capability and quality of commercial assays is insufficient and that the levels of free IgE identified may be too high.
You conclude that using these measurements to monitor therapy would result in an overdose.
We only want to mention that in contrast to your results, in our clinical samples, we found low levels of free IgE land higher levels of omalizumab.

It must be stated that the recovery ELISA IgE/omalizumab assay is a multiplex assay, which provides additional information about drug activity (omalizumab activity).

In contrast to other assays tested by the authors, the BioTeZ recovery IgE ELISA/omalizumab assay is a quantitative method that simultaneously provides three measurements for each sample: the concentration of free IgE, the degree of IgE neutralisation (activity), and the concentration of therapeutic antibody omalizumab still available. The IgE neutralisation ratio indicates how much IgE was bound in the serum by therapeutic antibodies – the “recovery” refers to the recovery of the IgE added to the sample as a reciprocal of the degree of neutralisation.

Figure 1a shows the recovery of IgE in relation to the concentration of the therapeutic antibody.
Figure 1b shows the degree of neutralisation of IgE (omalizumab activity) or the ability of the omalizumab IgE to bind as a function of the omalizumab level.

Figure 1a

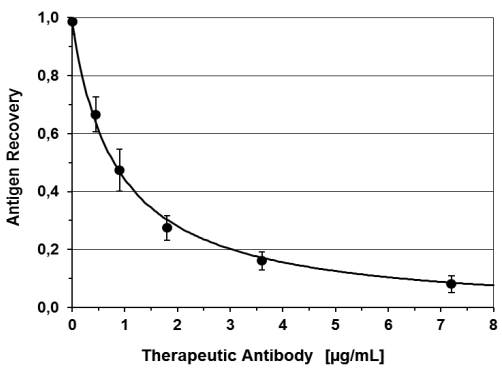
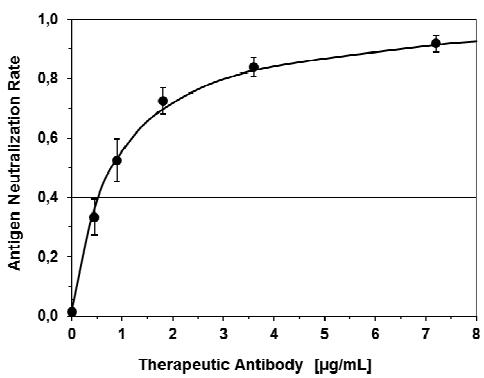


Figure 1b



The functions describe the interaction between the IgE and omalizumab biomolecules. The curve shows that the relative ability of omalizumab to neutralise the IgE decreases with increasing dose.

The BioTeZ recovery ELISA IgE/omalizumab assay is currently the only commercial measurement method that reflects the direct interaction between IgE and omalizumab. This parameter is ignored by the authors.

We are convinced that a parameter of the drug activity can prevent over- or under-dosage in asthma, urticaria, or psoriasis therapies with omalizumab – namely when it is seen in which IgE neutralisation area a patient is and where it is apparent that additional IgE neutralisation can be expected from a change in the dose.

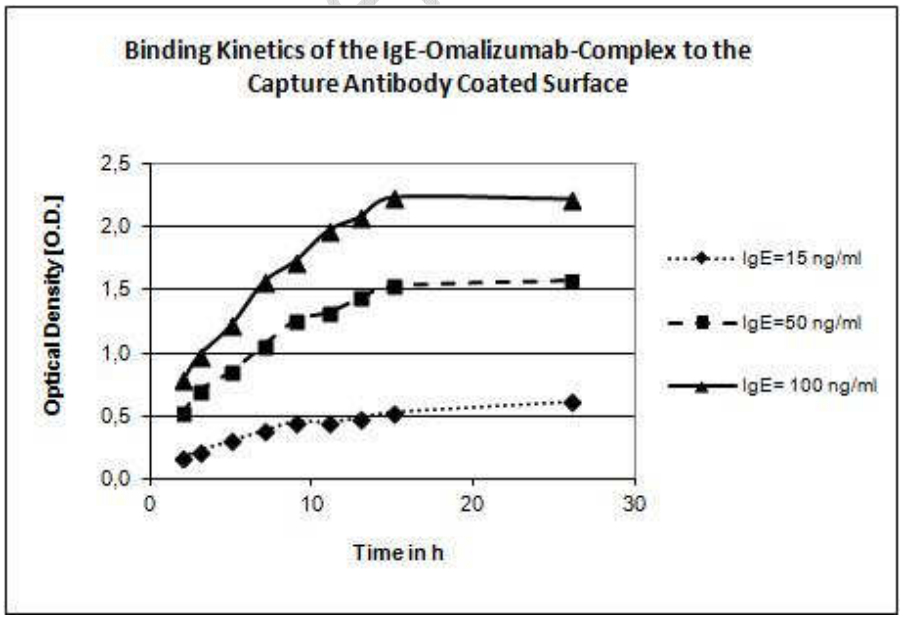
Table 1: below you will find an analysis of a serum sample that was measured with the recovery ELISA IgE/omalizumab assay. Doctors can match this information from data from the patient record e.g. applied dose, application intervals, and body weight.

free IgE	Recovery Value	IgE – Neutralisation rate (activity)	Omalizumab
17.0 ng/mL	3.2 %	96.8 %	28.0 µg/mL

The authors believe that the level of free IgE measured by the recovery ELISA assay was too high and suspect that this results from long incubation times and the high sample dilution.

For incubation, it should be noted that although the omalizumab-IgE complex quickly reaches equilibrium in solution, binding to the solid phase is merely delayed and occurs after 16 h. The experimental evidence is shown in Figure 2.

Figure 2: Binding Kinetik of IgE-Omalizumab-Complex to the capture antibody coated on the surface of a microplate with different IgE concentrations



In order to measure two analytes (IgE and omalizumab) in parallel with the recovery ELISA assay, both analytes must be in a measurable range. For this reason, native serum samples are measured in a dilution of 1:20. In the recovery ELISA assay for IgE and omalizumab, the measurement ranges were optimised by measuring native samples from patients undergoing omalizumab therapy and adjusting them to the clinically relevant range. The ranges of both analytes (IgE: 2.4–1210 ng/ml, omalizumab: 400–80,000 ng/ml) significantly differ from those of the Genentech in-house methods for which a significantly lower measurement range is given for both free IgE (2–150 ng / ml) and omalizumab (≥ 28 ng/ml). The Genentech omalizumab assay is also diluted 1:100.

As the authors appropriately write, comparing ELISAs with widely varying ranges as well as with different precision profiles and error distributions is always a source of deviations, which need to be analysed. We see no excessive source of error in the dilution selected during the recovery ELISA assay and are amazed at the poor reproducibility.

We have determined the inter-assay variance (Table 2), which is an important quality criterion of the assay. For this purpose, five native human serum samples were selected. Four of the five samples were from patients undergoing omalizumab therapy.

Table 2: Inter-Assay-Variance des *recovery*ELISA IgE/Omalizumab

Sample	Mean IgE (IU/mL)	CV (%)	Mean Omalizumab (µg/mL)	CV (%)
1 (n=12)	86.5	5.9	< LOQ	-
2 (n=12)	44.7	9.6	17.4	13.2
3 (n=12)	2.6	5.2	20.7	7.4
4 (n=12)	22.3	9.4	45.2	12.9
5 (n=12)	25.7	12.1	53.0	13.4

LOQ: Limit of Quantification; CV: coefficient of variation

We are deeply concerned by the reproducibility and false positives determined by the authors, which contradict our own quality management based measurements.
Our considerations range from errors caused by improper assay execution to methodological issues. In this context, we also question why the software developed to evaluate the recovery IgE ELISA/omalizumab assay was not mentioned by the authors. This software already includes a quality control and provides information on the accuracy of the experimental measurements.

A probable cause is the in vitro modification of the samples as well as the handling of the samples between analyses 1 and 2, which is not explained by the authors. It is critical that both different omalizumab-positive samples (in vivo samples) and omalizumab-negative samples from different patients were pooled with a native IgE level of 50–700 IU/ml and spiked with different amounts of omalizumab (in vitro samples). The term “in vivo samples” appears to be misleading.

It is assumed that IgE binds with omalizumab and that this binding process is crucially influenced by a number of factors such as incubation time, temperature, and the initial concentration of the components. Even when pooling native omalizumab-positive samples, there should be binding reactions after mixing, depending on the sample composition and how much unbound omalizumab

was included in the samples. The authors did not provide any further information about the mixing conditions of the samples or any supplements.

We would also like to note that results have not been presented for reproducibility with the other assays.

In Table 4 of the article, it would have been informative to show the measured values of the other assays, too. That could possibly explain why Figure 3 shows levels of free IgE that are above the measuring range of the Genentech assay.

With respect to the target level of < 50ng/ml IgE mentioned by the authors and the maximum allowed pre-treatment IgE level of 30–700 IU/ml, reference is made to current dosing recommendations for pre-treatment IgE up to 1500 IU/ml IgE (Table 3; Document WC 500 057 298 from EMA – Annex I – Summary of product characteristics; http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_Product_Information/human/000606/WC500057298.pdf), Last update 4 April 2014).

The publication on the comparison of different diagnostics is cause for discussion.

- Why should the desire of doctors for more information in case of treatment failure not be justified?
- Does personalised medicine not also entail a personalisation of the dose?
- Is the level free IgE during omalizumab therapy not relevant, although it is the target molecule and has a limit value < 50ng/ml, or it is because the therapeutic concentrations are saturated?

In our opinion, sound results can only be achieved through experiments with a transparent framework.

Unlike the authors, we believe that the BioTeZ *recovery* IgE ELISA/omalizumab assay is suitable for reliably measuring serum samples from patients during omalizumab therapy. Although we have not yet had any reasons to doubt this, we will conduct further tests.

Unfortunately, we cannot check the methods and results presented in the article because the assays and samples are not available.

We are available for further discussions and investigations.
We strongly welcome diagnostic methods that are suitable for personalised medicine, as we believe that this trend is becoming increasingly important – not only in the case of non-responders.

In addition to the development of highly innovative biologicals, the associated diagnostics should also be encouraged. BioTeZ is willing to meet this challenge and is always ready to cooperate.

Legend:

This is the pre-peer reviewed version of the following article: "Comment on Dana L. Baker et al. CMRO 2014; 1-10: printed May 2014, Vol. 30; No.5: 913-922) „Evaluation of two commercial omalizumab/free IgE immunoassays: implications of use during therapy", which has been published in final form in CMROJournal: Posted online on June 10, 2014. (doi:10.1185/03007995.2014.933099) (http://informahealthcare.com/doi/abs/10.1185/03007995.2014.933099)

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Transparency:
P.S. is the Inventor and patent holder of the recovery-ELISA.
G.B. is an independent medical expert and reviewer for approval as a medical device for equipment and diagnostic tools, e.g. for recovery-ELISA.

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References:
Patent: EP 06828568.3 (Strohner, Pavel) Immunoassay for the simultaneous immunochemical determination of an analyte (antigen) and a treatment antibody targeting the analyte in samples (Recovery immunoassay)

Strohner et al.: The recovery ELISA – a newly developed immunoassay for measurement of therapeutic antibodies and the target antigen during antibody therapy. Clin. Chem. Lab. Med.: 2012; 50, 1263-1269.