Letter to the Editor

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Reply to the letter of Katayev and Fleming

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To the Editor,

We appreciate that Katayev and Fleming have studied our recent review on indirect approaches for estimating reference intervals (RIs) [1]. In this review, we mentioned about 20 various indirect methods, including the method of Katayev et al. [2, 3]. This approach is a completely computerized solution for RI estimation, according to the authors based on Hoffmann’s method. In agreement with Jones and Horwitz [4] and Holmes and Burr [5], we raised some criticism regarding the approach of Katayev et al.

In their reply-to-the editor [6], Katayev and Fleming refused our criticism. However, their arguments are not convincing for several reasons.

(1) The authors presented figures without disclosing the source of their data. Thus, we have the impression that the data are unrealistic artificial distributions.

(2) “Haeckel et al. claims that the Hoffmann method is unable to discriminate between two superimposed distributions with one of them belonging to non-diseased subjects (main distribution) and another belonging to diseased subjects (secondary distribution)”.

In their reply, Katayev and Fleming present Figure 1A–C to prove that the Hoffmann approach is able to discriminate distributions. In fact, this Figure perfectly supports our statement. Figure 1A and C shows distributions with two local maxima. Between these maxima, the density lies clearly above zero. This means that both sub-distributions overlap considerably and consequently, there is no cutpoint which has all points from one distribution to the left and all points from the other distribution on the right. Therefore, the “main linear region” suggested by Figure 1B will either contain all “non-diseased” data or it will contain also “diseased” data. Both alternatives provide inadequate data to the next step of parameter estimation. The interval of x-values will be too large or too small and consequently the cumulative density function, from which parameters will be estimated, is inaccurate. The linear regression estimation procedure in refs. [2, 3] is not able to provide estimates from a truncated distribution (with some non-diseased subjects having values outside the “main linear region”) and is not robust against diseased data in the “main linear region”.

We write “main linear region” between quotation marks because, as shown quite nicely by Figure 1B, there is no linear region in the cumulative density function of a continuous distribution.

(3) “Haeckel et al. erroneously stated that we claimed to have adopted the Hoffmann approach without any changes”.

Our statement refers to ref. [2], where the authors declare on p. 182 “The Hoffmann indirect method for the derivation of RIs was programmed as originally described”, referring to the Hoffmann paper from 1963 as a reference. In their 2015 paper [3], the authors write “The new computerized Hoffmann’s statistical algorithm was completely redesigned from the previously published prototype”. However, the essential step of the parameter estimation is the same as in ref. [2], namely linear regression in a “main linear region”. The expansions in ref. [3] compared to ref. [2] refer mainly to data handling, to stratification, to an extended output, to the introduction of a Box-Cox transformation of the data, and to the introduction of a formal procedure for identifying the so-called “main linear region”. In that sense, the procedure is fully computerized.

The crucial component of the Hoffmann approach is plotting a cumulative distribution on probability paper in order to obtain a straight line for the “normal” component of the data. The importance of using probability paper is emphasized by Hoffmann himself on p. 154 in ref. [7], where he formulates “probability paper has separated the two components for us”. No plot on probability paper can be seen in ref. [2] or ref. [3], rather the title of ref. [3] says “no Longer a Probability Paper Method”. Instead, in ref. [2] and ref. [3] the authors fit a straight line to some portion of the untransformed cumulative distribution function (cdf). This is shown in Figure 1 in ref. [2] and in Figure 2 in ref. [3]. The difference between the Hoffmann plot on graph paper and
the Katayev et al. plot of the untransformed cdf is not an aesthetic one. It goes along with different calculations. Unfortunately, Katayev et al. failed to recognize that (i) a Gaussian cdf is nowhere linear and (ii) the correct line for estimating Gaussian parameters from the cdf passes the cdf point corresponding to a probability of 50% and having the slope given by the first derivative at this position. The Katayev regression line, which never coincides with the correct line, has a systematic bias in the slope estimate. This would have been avoided by transforming the cdf by using probability paper, as suggested by Hoffmann. Therefore, if the authors programmed the original Hoffmann approach, as they claim, their presented results stem from a different calculation.

(4) “Haeckel et al. stated that we do not use transformation of values”.

This statement refers to not transforming the cumulative distribution function as outlined in the previous paragraph.

(5) “Finally, Haeckel et al. are issuing conflicting statements … we provide compelling evidence that our calculations are not statistically different from published high quality direct reference interval studies”.

We do not agree with the statement that the RIs estimated by Katayev et al. always agree with data in the literature. In several cases the lower reference limits are outside the confidence intervals (Table 3 and Figure 3 in ref. [3]). The upper RLS for the author’s calculations and for the literature data do not overlap with measurand A, C and F. Furthermore, the author’s reference range were distinctly larger than the reference ranges taken from the literature for measurand B, C, D, E, F, G and K.

As we pointed out in our review, although the direct IFCC is the present gold standard, it has many disadvantages. We have also included a chapter on the verification of RIs established by indirect methods. In this chapter, we discussed the various problems when RIs are compared with each other. See also our publication in *Clinical Chemistry and Laboratory Medicine* [8].

**References**

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