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LETTER TO THE EDITOR: UNLIKELINESS LIMITS ESTIMATION

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One of the requirements concerning the working instructions for measurement procedures (or measuring systems) given in the subclause 5.5.3 of the standard ISO 15189:2007 (1) states that the clinical laboratory documentation should include the reportable interval of examination results, and in the subclause B.5.6 of the Annex B states that, for each examination procedure, a range [interval] of values should be predefined to detect *absurd* or *impossible* results. Thus, any laboratory seeking accreditation for compliance with the mentioned standard shall establish the limits for these intervals.

On the other hand, the same standard, in the subclause 5.7.1, states that authorized personnel shall review systematically the results of examinations in the post-examination phase. One way of performing this systematic review is the plausibility control (2), using alert limits, among other tools, in order to detect *doubtful results*. Some of these doubtful results detected using this way could be results with a very low probability of belonging to the patient, here named *unlikely results.*

Bearing in mind these two objectives, it is advisable to set a criterion to estimate *unlikeliness limits* which will define the unlikely results, aside from the clinical implication of these results.

As there are not scientifically rigorous procedures to set unlikely limits, the establishment of such limits will be more or less arbitrary. The current text discusses several strategies that can be used to estimate the unlikeliness limits as well as the problems appeared in this estimation. The manuscript describes and compares different methods for the definition of the reportable interval of an examination procedure.

In order to establish the unlikeliness limits of several biological quantities measured in the clinical laboratory of the Hospital Universitari de Bellvitge (Table 1), for each quantity, the measured values reported during years 2006 and 2009, and stored in the laboratory information system Omega 3000 (Roche Diagnostics España S.L., Sant Cugat del Vallès, Catalonia, Spain) were used, as long as 10 000 data or more were available.

Among the different procedures proposed to estimate the unlikeliness limits, it is necessary to find out which one, despite of its arbitrariness, allows the establishment of these limits taking care that the number of unlikely results be reasonable under a professional point of view.

One of the proposed procedures to get a limit is based in the estimation of fractiles beyond which will be very unlikely to find a result, although the choice of this fractile is completely arbitrary (3).

Another proposed procedure is based on considering unlikely any result outside the range defined by the higher and the lower of the cumulated reported (validated) results, after excluding possible outliers (4).

On the other side, if the definition of unlikely value is considered under a statistical point of view, this definition is equivalent to the definition of outlier (5). Thus, a statistical test for outlier detection may be adapted to estimate unlikeliness limits.

In accordance with the Dixon's test for outliers detection (5), in a series of results (containing outliers) sorted from lowest to highest:

x_n is an outlier when $x_n - x_{n-1} > (x_n - x_1)/3$

x_1 is an outlier when $x_2 - x_1 > (x_n - x_1)/3$

Thus, any result being $\leq x_1$ or $\geq x_n$ will be considered outlier and, consequently, x_2 will be the first non outlier value and x_{n-1} the last non outlier value.

Therefore, in a series of results sorted from lowest to highest, the value of the first hypothetical outlier in the right side, x_n, can be calculated as follows:

$$x_n - x_{n-1} = (x_n - x_1)/3 \;\; ; \;\; 3x_n - 3x_{n-1} = x_n - x_1 \;\; ; \;\; x_n = (3x_{n-1} - x_1)/2$$

The hypothetical first outlier in the left side, x_1, is calculated similarly:

$$x_2 - x_1 = (x_n - x_1)/3 \;\; ; \;\; 3x_2 - 3x_1 = x_n - x_1 \;\; ; \;\; x_1 = (3x_2 - x_n)/2$$

Thus, the hypothetical first outlier in the left side corresponds to the lower unlikeliness limit and the first outlier in the right side corresponds to the upper unlikeliness limit.

It is well known that, in order to be sure that the estimation of limits is acceptable, it is very important that all the measured values used belong to the selected population, that is, they are not outliers. However, there is not literature enough with proper information regarding the most appropriate procedure to detect outliers, especially in populations with such large number of data and with non Gaussian distribution as the used in the current study.

In this work, we selected a modification of the Dixon's test, modified according to Reed, Henry, and Mason (5, 6), for the detection of outliers, because, despite presenting some limitations (masking outliers when instead of a single outlier result is a collection of data that is not part of the sample), this test is one of the recommended criterion by the American organization *Clinical and Laboratory Standards Institute (CLSI)* for the establishment of reference values (7), which is a situation with some analogy with that presented in this article.

In Table 1, different unlikeliness limits for each biological quantity considered are showed. These limits have been estimated using the three strategies mentioned above (fractile, lowest and highest non outlier value, estimation of the minimum hypothetical outlier value using the Dixon's inequation). We have to take into account that increasing the number of available data means that the established unlikeliness limits will be more reliable, so, it is reasonable to review them once a year.

It should be remarked that for kinds of quantity related with fractions (substance fraction, mass fraction, volume fraction, etc.), the Dixon's inequation is not applicable. Because the actual measurement results belong to the interval between 0 and 100 (or 0 and 1), and the Dixon's inequation may give unlikeliness limits outside this range.

Moreover, this strategy will not be applicable in the estimation of the lower unlikeliness limit, particularly in such cases where the corresponding numerical value is close to 0, and after applying the corresponding equation, the theoretical outlier values will correspond to a negative value. Nevertheless, bear in mind that in most cases, there are biological quantities for which ones the establishment of a lower unlikeliness limit makes no sense. There are biological quantities that in some patients have values below the detection limit of the measurement procedure (and

therefore must be reported with the relational operator "≤" followed by the detection limit value and the corresponding unit).

The Dixon's inequation strategy, although provides a reasonable number of unlikeliness values, it presents some limitations. In fact, the main problem of this approach is that it can produce results so abnormal to be completely useless for the scope of avoiding inappropriate reporting; thus, an unlikeliness limit found following this approach may be a value incompatible with life (although it is very difficult to find information about which values are incompatible with life).

Probably, as the setting of unlikeliness limits is, by definition, arbitrary, any of the three approaches could be equally valid.

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Table 1. Unlikeliness limits estimated for several biological quantities according to various strategies mentioned in the text. [*n* = number of data used for the estimation; *x* = fractile; *LL*= lower non-outlier limit; *UL* = upper non-outlier limit.]

Quantity* [unit]	*n*	Fractiles				*LL* and *UL* Non-outliers		Lowest and highest hypothetical outliers	
		$x_{0.005}$	$x_{99.995}$	$x_{0.0005}$	$x_{99.9995}$	*LL*	*UL*	Lowest	Highest
P—Alanine aminotransferase; cat.c. [µkat/L]	63701	0.1	32	0.1	77	0.1	103	-	155
P—Albumin; mass c.(CRM 470) [mg/L]	16383	14	53	11	55	10	57	-	80
P—Alkaline phosphatase; cat.c. [µkat/L]	49537	0.4	39.1	0.3	75.5	0.3	83.6	-	125.2
P—Aspartate aminotransferase; cat.c. [µkat/L]	31990	0.11	423	0.1	189	0.1	289	-	434
B—Basophils; num.c. [10^9/L]	13343	0	0.5	0	1.1	0	1.1	-	1.6
P—Bilirubin (ester); subst.c. [µmol/L]	37745	1	644	1	842	1	918	-	1376
P—Bilirubin; subst.c. [µmol/L]	34147	2	709	1	949	1	1164	-	1746
P—Calcium(II); subst.c. [mmol/L]	65530	1.4	3.4	1.1	3.8	1	3.8	-	5.2
P—Carcinoembryonic antigen; mass c. [µg/L]	10053	0	19499	0	23236	0	24229	-	36343
U—Chloride; subst.c. [mmol/L]	18901	4	270.6	2	320	2	352	-	527
Pt(U)—Chloride excretion; subst.rate(24h) [mmol/d]	19459	1	634	1	1121	1	1474	-	2211
P—Cholesterol; subst.c. [mmol/L]	55055	1.2	13.8	0.7	23.6	0.5	25.1	-	37.3
P—Cobalamin; subst.c. [pmol/L]	16091	37	1476	22	1476	22	1476	-	2203
P—C reactive protein; mass c.(CRM 470) [mg/L]	52235	0	489	0	555	0	751	-	1127
P—Creatininium; subst.c. [µmol/L]	65478	20	1141	19	1342.6	19	1548	-	2312
Pt(U)— Creatininium excretion; subst.rate(24h) [mmol/d]	22015	0	42	0	67	0	69	-	104
B—Eosinophils; num.c. [10^9/L]	13343	0	2.3	0	3.7	0	4.1	-	6.1

Quantity* [unit]	*n*	Fractiles				*LL* and *UL* Non-outliers		Lowest and highest hypothetical outliers	
		$x_{0.005}$	$x_{99.995}$	$x_{0.0005}$	$x_{99.9995}$	*LL*	*UL*	Lowest	Highest
B—Erythrocytes; num.c. [10^{12}/L]	13371	2.0	7.1	1.7	7.9	1.7	8.0	-	11.2
P—Ferritin; mass c. [µg/L]	59907	3	15388	1	68599	1	122312	-	183467
P—α-Fetoprotein; mass c. [µg/L]	20798	1	267565	1	542299	1	658310	-	987465
P—Folate; subst.c. [nmol/L]	14188	4.7	45.4	4.0	45.4	3.9	45.5	-	66.3
P—Glucose; subst.c. [mmol/L]	62741	1.9	24	1.1	36.5	0.8	50.0	-	74.6
P—γ-Glutamyltransferase; cat.c. [µkat/L]	65513	0.1	51.6	0.0	77.5	0.0	125.3	-	187.9
P—HDL cholesterol; subst.c. [mmol/L]	16007	0.1	3.7	0.1	4.4	0.1	4.5	-	6.7
B—Hemoglobin; mass c. [g/L]	13371	55	193	34	231	14	240	-	353
Hb(B)—Hemoglobin A1c; subst.fr. [mmol/moL]	13596	3.2	16.4	3.2	18.2	3.1	18.4	-	26.0
Pla—Homocysteine; subst.c. [µmol/L]	13970	2.2	124.2	2.0	192.2	2.0	195.0	-	291.5
P—Immunoglobulin G; mass c. [mg/L]	11690	155	92029	87	106078	57	109000	-	163472
P—Immunoglobulin M; mass c. [mg/L]	10664	18	66410	15	91288	11	93100	-	139644
P—Iron; subst.c. [µmol/L]	60164	1	63	1	79	0	87	-	130
P—L-Lactate-dehydrogenase; cat.c. [µkat/L]	60597	1	91	0	166	0	248	-	287
B—Leukocites; num.c. [10^9/L]	13371	0.0	83	0	118	0	122	-	183
B—Lymphocytes; num.c. [10^9/L]	13342	0	70	0	111	0	116	-	174
Lymphocytes(B)—Lymphocytes $CD3^+CD4^+$; num.fr. [%]	12842	1	69	0	74	0	79	-	118
Lymphocytes (B)— Lymphocytes $CD3^+CD8^+$; num.fr. [%]	12841	2	87	0.64	94.72	0	96	-	144
P—Magnesium(II); subst.c. [mmol/L]	26533	0.2	2.6	0.1	3.0	0.0	4.2	-	6.4
Pla—Mycophenolate; mass c. [mg/L]	15931	0	10	0	13	0	14	-	22
P—β2-Microglobulin; mass c. [mg/L]	17112	0	75	0	85	0	98	-	148

Quantity* [unit]	*n*	Fractiles				*LL* and *UL* Non-outliers		Lowest and highest hypothetical outliers	
		$x_{0.005}$	$x_{99.995}$	$x_{0.0005}$	$x_{99.9995}$	*LL*	*UL*	Lowest	Highest
B—Monocyte; num.c. [10^9/L]	13341	0	3.4	0	4.5	0	4.7	-	7.0
B—Neutrophils; num.c. [10^9/L]	13343	0	54	0	78	0	84	-	127
P—Phosphate; subst.c. [mmol/L]	59903	0.2	3.9	0.2	4.8	0.1	5.9	-	8.8
B—Plattelets; num.c. [10^9/L]	13359	3	1172	1	28456	1	3280	-	4920
P—Potassium ion; subst.c. [mmol/L]	37938	2.4	7.2	1.9	7.9	1.8	8.3	-	11.5
Pt(U)—Potassium ion excretion; subst.rate(24h) [mmol/d]	21200	1	241	0	581	0	601	-	901
P—Protein; mass c. [g/L]	57157	31	119	22	130	21	141	-	201
Pt(U)—Protein excretion; mass rate(24h) [g/d]	27236	0.0	23.9	0.0	45.4	0	54.4	-	81.6
P—Rheumatoid factors; subst.c.arb.(WHO 64/2) [kint.u./L]	11012	7	2092	6	3244	5	3274	-	4908
P—Sodium ion; subst.c. [mmol/L]	37934	115	160	109	167	108	174	**76**	207
B—Tacrolimus; mass c. [µg/L]	10752	1.5	29.1	1.2	30.0	1.2	30.0	-	44.4
P—Transferrin; subst.c.(CRM 470) [µmol/L]	32626	5	63	3	73	3	84	-	125
P—Triglyceride; subst.c. [mmol/L]	59333	0	23	0	53	0	111	-	117
P—Urate; subst.c. [µmol/L]	58669	38	961	18	1308	12	1378	-	2061
P—Urea; subst.c. [mmol/L]	65516	1	57	1	77	1	86	-	128
U—Urea ;c.subst. [mmol/L]	14238	10	633	9	657	9	657	-	981
Pt(U)— Urea excretion; subst.rate(24h) [mmol/d]	20524	2	1157	0	1596	0	1611	-	2416

*Quantities are described according to IFCC and IUPAC: B = blood; cat.c. = catalytic concentration; CRM = certificate reference material; mass c. = mass concentration; P = plasma; subst.c. = substance concentration]