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USE OF REFERENCE VALUES FOR THE PROFICIENCY TEST SCHEME IN THE MEASUREMENT OF GLUCOSE AND CHOLESTEROL IN HUMAN SERUM

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Abstract: The first proficiency testing round 630-IL-1002, was carried out with a Reference Material DMR-180a with reference values obtained by using GC-IDMS methods, in which glucose, cholesterol and creatinine were measured. The serum pool was obtained from blood donors and all the analytes were at the normal concentration in Mexican population. The laboratories participants used different field methods to measure the analytes. The Mexican compulsory standard NOM-064-SSA1-1993 "specifications for equipments IVD" requests <5% for reproducibility and accuracy of IVD equipments. The results obtained by field laboratories in the proficiency testing round are compared to the reference value and uncertainty provided by National Metrology Institute (CENAM). The quality of measurements are dependent not only on the laboratory competence but also on the methods used by those commercially available IVD kits. It is concluded that quality assessment of measurements in clinical laboratories should be critically evaluated by using stable and certified reference materials.

Keywords: reference values, proficiency test, certified reference materials.

1. INTRODUCTION.

In Mexico, the clinical laboratories try to establish traceability in their measurements and they work for accreditation according to NMX-EC-17025-IMNC-2000 "General requirements for the technical competence by test and calibration laboratories", then they need to show traceability to the National Measurement Institute. By the time of the comparison the clinical laboratories quantify health status markers by using calibration curves prepared by commercially available (bovine for example) with no evidences of traceability to SI units and do not report uncertainty value, so, was important to demonstrate that the use of CRMs could give support to metrological aspects in clinical area and PT schemes.

A proficiency testing round was organized to measure glucose, cholesterol, creatinine and calcium in human serum. A CRM was used as a blind sample for the participants in the PT scheme. Four Mexican clinical associations that represent clinical laboratories were invited to participate in the PT, and each of them invited twenty laboratories established in different parts of the country. Some public health laboratories were also invited in order to obtain representative samples of the clinical laboratories in the country. 46 laboratories from different regions of Mexico have reported results.

Whit this PT was tried to identify the different analytical methods used in Mexico to measure the analytes mentioned above, to know about the measurement capability of the laboratories and also to give traceability to these laboratories. With this PT we also want to help them to identify the source of possible bias in the measurements by the methods employed and reduce the variation obtained as much as possible. Other intention was to form a network of laboratories for clinical measurements, to collaborate with calibrators manufactures to integrate them into CENAM's MRTC program to provide traceability in clinical measurements and to guarantee to the population that clinical analysis services are reliable in the country and comparable to those of other countries.

The main body of the abstract should be divided into sections. For greater clarity the standard model may be used (not demanding), composed by Introduction, Purpose, Methods, Results, Discussion and Conclusions. Each section may be divided in items. The initial section (Introduction) should present the problem and describe the state of art in the area, as well as the proposed solution and their merits. The following sections (Purpose, Methods and Results) should describe in details the methods and procedures used in the research and the obtained (experimental or simulated) results. In the following section (Discussion), the obtained results should be compared with the ones obtained by other authors, discussing the advantages and limitations of the proposed solution. In the final section (Conclusion) the results , the restrictions to the application of the method and the possibility of applying the results, should be presented.

Acknowledgments can be expressed briefly after the main body of the abstract. At the end, the list of bibliographic references must be added.

2. METHODS

2.1. Sample preparation

The human serum material was colleted from the blood donors in the transfusion center (Blood Bank) at Querétaro city taken in account all the standards required for that purpose in order to guaranty the safety use of the reference material. The DMR 180a was prepared at CENAM. To prepare the serum pool each serum donor tube was placed into a sterile glass, after that the pool, was stirred gently for 15 min, then gentamicine sulfate was added as an antibacterial agent and again stirred for 15 min. The pH was measured and finally the pool was filtered and ampouled in 2,0 ml glass ampoules. For each ampoule we dispensed 1 ml of serum with automatic pipette and the ampoule was sealed with flame.

The samples were stored at -80 ° C until use.

The analytes of interest were certified by using isotopic dilution mass spectrometry, the same method used to demonstrate the CENAM capability of measurement for glucose reported at the BIPM web page. On the other hand this sample fulfills the requirements for homogeneity and stability tests for a CRM.

2.2. Organization of the intercomparison

Two ampoules of the DMR 180a containing 1 mL of frozen human serum and the protocol of measurement were sent to each participating laboratory. The laboratories were requested to return the results of five measurements for each analyte in each ampoule one month lather. The results were evaluated and discussed with the laboratories participants in the National Clinical Sectorial Seminar.

3. RESULTS AND DISCUSSION

In this paper only the results of glucose [1] and cholesterol [2] are reported. The largest intralaboratory variation (15%) in terms of RSD and the largest bias (42 %) with respect to the reference value were reported for cholesterol, while those for glucose were 14% and 20 %, respectively. In Figs. 1a and 1b, individual results are plotted for glucose and in Figs. 2a and 2b for cholesterol. Also the mean square error (MSE) for each laboratory were calculated [3]. MSE is one of the criteria that permit to evaluate the capability of measurement of the participant laboratories. In Table 1 are shown the results of MSE obtained for all laboratories for glucose and Table 2 for cholesterol.

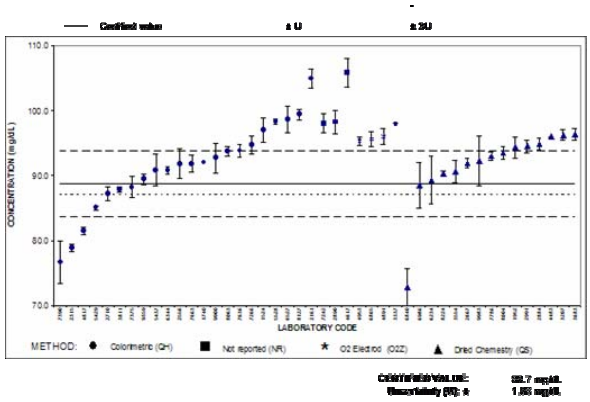

Fig. 1a. Mean value and standard deviation for glucose concentration in sample 1

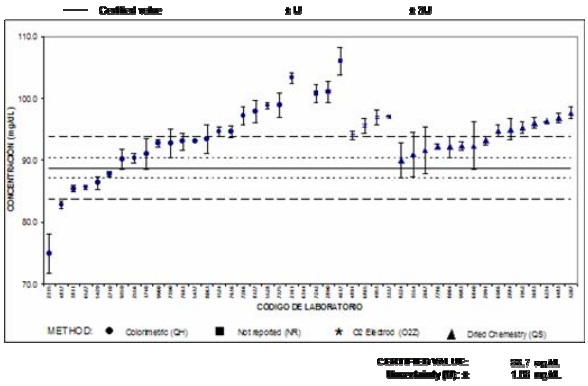

Fig. 1b. Mean value and standard deviation for glucose concentration in sample 2

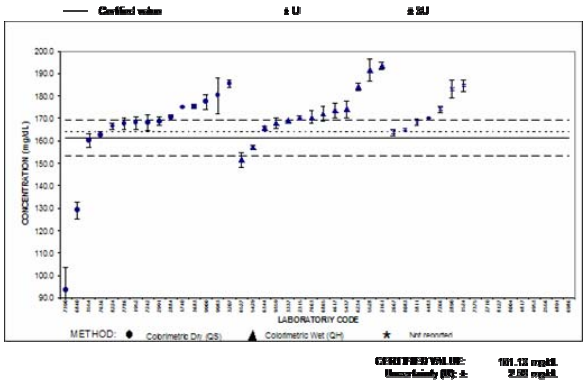

Fig. 2a. Mean value and standard deviation for cholesterol concentration in sample 1

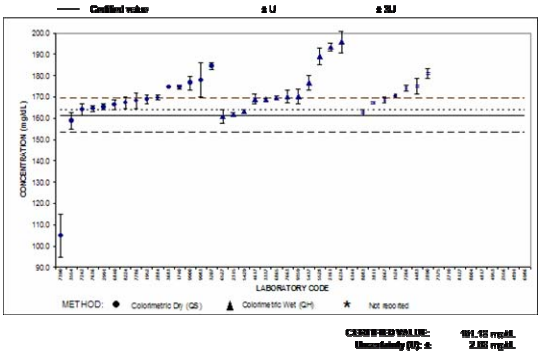

Fig. 2b. Mean value and standard deviation for cholesterol concentration in sample 2

Three different methods: colorimetric wet (CH), colorimetric dry (QS) and O2 electrode (O2Z), were reported by participating laboratories, as it is shown in Figs. 1a and 1b and Table 1 the method dependence of the results for both samples can be summarized as follows: 1. Colorimetric dry gives consistent results (except one laboratory) with some bias to higher values. 2. Colorimetric wet gives results with high dispersion of the results among laboratories. 3. O2 electrode gives less dispersion but bias to higher value. Thus, in 49% of the cases they reported at least for one sample a value with bias to higher value from the reference value larger than 5%, which is the criteria of Mexican standard.

Cholesterol was measured by field laboratories by using two different methods, colorimetric wet and dry, two laboratories did not report which method they used. As it is shown in Fig 2a and 2b and Table 2. In cholesterol results, both methods tend to give bias to higher values. From the laboratories that measured cholesterol 19% reported one of the two values with bias from reference value bigger that 5% and 33% of the laboratories reported both values with bias from the reference value bigger that 5%. Thus, in 51 % of the cases they reported at least for one sample a value with bias from the reference value bigger than 5%. For intra-laboratory variation 7% of the laboratories obtained a RSD larger than 5%.

Table 1. Results obtained for glucose sample 1.

LAB	CODE	MEAN	Std dev	BIAS	ECM	MÉTHOD
20	3811	87.80000	0.42164	0.90000	0.99387	QH
35	9359	89.43000	0.83273	0.73000	1.10740	QH
12	8224	90.30000	0.48305	1.60000	1.67133	QS
6	7375	88.20000	1.61933	0.50000	1.69476	QH
13	2710	87.20000	1.03280	1.50000	1.82117	QH
43	6344	90.80000	0.63246	2.10000	2.19317	QH
15	3554	90.60000	1.71270	1.90000	2.55799	QS
27	5437	90.80000	2.44040	2.10000	3.21956	QH
24	2667	91.90000	0.73786	3.20000	3.28397	QS
44	1740	92.00000	0.00000	3.30000	3.30000	QH
25	7663	91.80000	1.31656	3.10000	3.36799	QH
41	6886	88.50000	3.53553	0.20000	3.54119	QS
4	5429	85.06000	0.33731	3.64000	3.65560	QH
33	6234	89.30000	3.68330	0.60000	3.73184	QS
34	2556	91.77000	2.29495	3.07000	3.83297	QH
1	7786	92.99000	0.68710	4.29000	4.34468	QS
22	9900	92.70000	2.26323	4.00000	4.59589	QH
16	8004	93.50000	0.97183	4.80000	4.89739	QS
40	8063	93.75000	0.70711	5.05000	5.09926	QH
26	7636	93.80000	0.91894	5.10000	5.18213	QH
5	9983	92.30000	3.83116	3.60000	5.25716	QS
10	1952	94.33333	1.65831	5.63333	5.87235	QS
37	2991	94.50000	0.97183	5.80000	5.88085	QS
18	7266	94.70000	1.41814	6.00000	6.16532	QH
17	2884	94.90000	0.87560	6.20000	6.26152	QS
28	4953	95.30000	0.67495	6.60000	6.63442	O2Z
31	6865	95.60000	1.17379	6.90000	6.99913	O2Z
19	4817	81.50000	0.52705	7.20000	7.21926	QH
36	4891	96.00000	1.24722	7.30000	7.40578	O2Z
11	3207	96.20000	0.78881	7.50000	7.54137	QS
8	3683	96.30000	0.94868	7.60000	7.65898	QS
21	1524	97.00000	1.82574	8.30000	8.49843	QH
32	7242	98.00000	1.49071	9.30000	9.41872	NR
3	5528	98.31000	0.45326	9.61000	9.62068	QH
39	2890	98.20000	1.75119	9.50000	9.66006	NR
9	2315	78.90000	0.56765	9.80000	9.81643	QH
23	6527	98.60000	2.01108	9.90000	10.10220	QH
14	8127	99.40000	0.84327	10.70000	10.73318	QH
42	7390	76.70000	3.23351	12.00000	12.42801	QH
30	6840	72.85000	2.85784	15.85000	16.10558	QS
7	2161	104.90000	1.44914	16.20000	16.26469	QH
29	4617	105.80000	2.20101	17.10000	17.24107	NR

Table 2. Results obtained for cholesterol sample 1.

LAB.	CODE	MEAN	Std dev	BIAS	ECM	METHOD
26	7636	162.60000	1.34990	1.42000	1.95924	QS
24	2667	163.80000	1.31656	2.62000	2.93219	NR
15	3554	160.00000	2.98142	1.18000	3.20644	QS
40	8083	165.00000	0.53452	3.82000	3.85722	NR
4	5429	157.16000	0.73364	4.02000	4.08639	QH
43	6344	165.70000	0.82327	4.52000	4.59436	QH
12	8224	166.50000	1.50923	5.32000	5.52993	QS
1	7786	167.70000	2.45176	6.52000	6.96574	QS
35	9359	167.90000	2.29541	6.72000	7.10122	QH
10	1952	168.00000	2.82843	6.82000	7.38325	QS
20	3811	168.50000	1.35401	7.32000	7.44417	NR
37	2991	168.60000	1.89737	7.42000	7.65875	QS
32	7242	168.30000	3.49762	7.12000	7.93270	QS
9	2315	170.20000	1.03280	9.02000	9.07894	QH
17	2884	170.50000	1.08012	9.32000	9.38238	QS
25	7663	170.60000	2.95146	9.42000	9.87155	QH
23	6527	151.60000	3.33999	9.58000	10.14554	QH
31	6865	172.10000	3.41402	10.92000	11.44124	QH
29	4617	173.60000	3.23866	12.42000	12.83531	QH
18	7266	174.10000	1.37032	12.92000	12.98247	NR
27	5437	174.10000	3.87155	12.92000	13.48760	QH
44	1740	175.00000	0.00000	13.82000	13.82000	QS
8	3683	175.30000	0.94868	14.12000	14.15183	QS
22	9900	177.40000	3.16930	16.22000	16.52673	QS
5	9983	180.20000	8.06639	19.02000	20.65979	QS
39	2890	183.10000	3.84274	21.92000	22.25428	NR
33	6234	184.00000	1.63299	22.82000	22.87835	QH
21	1524	184.70000	2.49666	23.52000	23.65214	NR
11	3207	185.60000	1.57762	24.42000	24.47091	QS
3	5528	191.70000	4.98767	30.52000	30.92165	QH
7	2161	193.40000	1.50555	32.22000	32.25516	QH
30	6840	129.00000	3.85861	32.18000	32.41051	QS
42	7390	93.50000	10.14615	67.68000	68.43630	QS

3. CONCLUSION

Based on the criteria established in the Mexican standard NOM-064-SSA2-1993, 48% of the IVD equipments used to measure cholesterol and 49 % used to measure glucose by field laboratories participants in the PT are out of specifications for accuracy, and 7 % for reproducibility of both cholesterol and glucose.

By using a reference value, it was demonstrated that most of the methods employed by field laboratories had bias to higher values and the dispersion is method dependent.

The use of certified reference materials is recommended to field laboratories to calibrate their instruments.

It is concluded that proficiency testing with reference value is essential for the evaluation of technical competence of clinical laboratories as well as calibrant quality.

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