ACTA IMEKO
ISSN: 2221-870X
June 2026, Volume 15, Number 2, 1 - 5

Screening mercury (Hg) presence in Philippine milkfish (*Chanos chanos*) using total reflection X-ray fluorescence (TXRF)

Aldrin Jan E. Tabuso[1], John Kenneth C. Valerio[1], Anton John D. Rocha[1], Alleni T. Junsay[1], Kim Christopher C. Aganda[1]

[1] *Department of Science and Technology, Industrial Technology and Development Institute (DOST-ITDI), Bicutan, Taguig City, Philippines*

ABSTRACT
In this study, a method for the analysis of mercury (Hg) content in Philippine milkfish (*Chanos chanos*) using Total Reflection X-ray Fluorescence (TXRF) spectrometry was validated using Philippine Reference Material (PRM) 2002-As, Cd, Hg & Pb in Milkfish, produced by the DOST-ITDI National Metrology Division, which resulted in a recovery ranging between 93 % and 104 %. Additionally, the relative standard deviation (RSD) of the collected dataset is lower than 10% and the expanded uncertainty is 0.08 ppm, showing the method's precision and consistency. For confirmation, DORM-5 was analyzed, resulting in 98.885 % Hg recovery, further supporting the capability of the TXRF analysis. The method was applied to milkfish samples harvested from different areas around Laguna de Bay to screen for the presence of Hg contamination. The dorsal meat of each milkfish sample was separated, dried, and crushed into smaller particles for TXRF analyses. The analyses showed that no mercury was detected in the milkfish samples which indicates that the milkfish from Laguna de Bay are free from harmful levels of Hg contamination.

Section: RESEARCH PAPER

Keywords: fish; heavy metals; total reflection x-ray fluorescence (TXRF); toxic elements

Citation: A. J. E. Tabuso, J. K. C. Valerio, A. J. D. Rocha, A. T. Junsay, K. C. C. Aganda, Screening mercury (Hg) presence in Philippine milkfish (Chanos chanos) using total reflection X-ray fluorescence (TXRF), Acta IMEKO, vol. 15 (2026) no. 2, pp. 1-5. DOI: 10.21014/actaimeko.v15i2.2300

Section Editor: Leonardo Iannucci, Politecnico di Torino, Italy

Received January 15, 2026; **In final form** June 2, 2026; **Published** June 2026

Funding: This work was supported by Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD).

Corresponding author: Kim Christopher Aganda, e-mail: kccaganda@itdi.dost.gov.ph

1. INTRODUCTION

In the 2023 Philippine Fisheries Profile prepared by the Bureau of Fisheries and Aquatic Resources (BFAR), Filipinos were reported to consume mostly fish and fishery/aquatic products, alongside rice and rice products. The average Filipino consumes 63.3 % more fishery products than meat products, and 205.9 % more than poultry products. Among these products, seaweed is the most widely produced aquatic product by volume, followed by tuna and milkfish [1], [2].

According to the BFAR, milkfish (*Chanos chanos*) is considered one of the most significant fish species farmed in the Philippines, because it can be cultivated in freshwater, brackish water, and certain marine environments [3]. The country's inland waters, which include lakes, rivers, swamplands, and reservoirs, cover about 500,000 hectares, of which approximately 90,000 hectares comprise Laguna de Bay, the largest lake in the Philippines [4]. Since the development of fish farms in Laguna de Bay in the early 1970s, the lake has provided livelihoods and aquatic resources [5]. However, due to the lake's proximity to densely populated areas, such as Metro Manila, Rizal, and Laguna, population growth, urbanization, and industrialization have contributed to the decline in water quality. Activities around the lake may increase the risk of contaminant accumulation, including heavy metals such as mercury (Hg) [4].

Mercury (Hg) is a naturally occurring element with a high atomic weight and a density at least five times greater than that of water [6], [7]. It is recognized as a major contributor to water pollution due to its toxicity, which can harm the lungs, kidneys, skin, and eyes, and affect the nervous, digestive, and immune systems [8]. Mercury levels in aquatic ecosystems are influenced by existing Hg in soils, and by Hg released through anthropogenic activities such as fossil fuel and coal combustion, gold mining, metal smelting, cement production, and industrial discharges. Organisms exposed to Hg-contaminated

environments may experience bioaccumulation, which is influenced by their growth rate; rapid growth may dilute Hg concentrations in tissues, while a short lifespan limits bioaccumulation over time. Since Hg cannot be degraded, it accumulates throughout the food chain, and increasing concentrations may pose health risks to consumers [9], [10].

Recently, X-ray fluorescence (XRF) spectrometry has been reported as one of the most effective and widely used techniques for determining heavy metals because of its non-destructive nature, rapid analysis, and capability for continuous measurements [11]–[13]. In particular, total reflection X-ray fluorescence spectroscopy (TXRF) is a specialized and well-established analytical technique used for multi-element determination in various samples, particularly liquids and small powder samples [14]. The X-rays used in TXRF produce fluorescence characteristic of each element, and the intensities of these signals are proportional to the concentrations of the elements in the sample [15]. This method allows concentration determination with minimal matrix effects, provides high sensitivity down to the ppb level, and requires only a small amount of sample (µg or µL) for analysis [16], [17]. In this study, a developed TXRF analytical method will be used to screen for Hg contamination in Philippine milkfish samples collected from different areas around Laguna de Bay. The results will be evaluated based on the World Health Organization (WHO) guidelines on safe Hg consumption levels, to determine whether the milkfish samples are safe for consumption.

2. METHODOLOGY

2.1. Reagents and materials

The TXRF instrument used for the analysis is the Bruker S2 Picofox, a compact and portable TXRF spectrometer designed for ultra-trace element analysis. It uses 30-mm-diameter quartz discs as sample carriers. All glassware, including the quartz discs, was cleaned by submerging it in a 5 % RBS 50 solution, a special detergent for glassware, then in a 20 % nitric acid bath.

For sample preparation, an octylphenol ethoxylate (Triton X-100) solution was used to suspend solid particles, and the internal standard was 1000 mg/L yttrium (Y), an element not typically found in milkfish. Yttrium is also used as an inert marker in fish digestibility studies because it is indigestible [18]. In addition to the actual milkfish samples, Philippine Reference Material 2002-As, Cd, Hg & Pb in Milkfish (PRM-2002) was also used for method development. This reference material is made from dried milkfish meat spiked with heavy metals, including arsenic, lead, mercury, and cadmium [19].

Figure 1. Milkfish sampling areas.

Table 1. Parameters set for the analysis of PRM-2002 using TXRF.

Parameter	Unit	Value
X-ray voltage	kV	50
X-ray current	µA	1000
Duration of scan	s	2000
Amount of IS (Y)	µL	10
Hg sensitivity factor	-	0.52
Y sensitivity factor	-	1.780218

The actual Philippine milkfish samples were harvested from different areas around Laguna de Bay, as shown in Figure 1, during their respective harvest seasons. The dorsal meat of the milkfish was used in the study since it is the most commonly consumed part of the fish.

2.2. Sample preparation

To prepare the samples, dorsal meat from the fish was collected, freeze-dried, and ground into fine particles. Then, 0.1 g of the solid sample was weighed on an analytical balance and mixed with 10 µL of a 1000 mg/L yttrium standard and 5 mL of a 1 % (v/v) Triton X-100 solution. The samples were placed in a sonicator at 5-minute intervals at least three times, to break up clumps in the solid sample. The samples were also agitated, using a vortex shaker to ensure that the solid particles were suspended in the liquid, before immediately transferring 10 µL onto a siliconized quartz disc. The samples were then allowed to air-dry until no visible liquid remained, resulting in a thin layer of solid particles, approximately 1 cm in diameter, deposited at the centre of the disc.

2.3. TXRF parameters

The analysis was set to 2000 seconds to increase the count rate for each element and improve the quantification of elements present in low concentrations. The calibration constants, also referred to as sensitivities, for the target element (Hg) and the internal standard (Y) are listed in Table 1, along with other analytical parameters.

3. RESULTS AND DISCUSSION

3.1. Calculation of concentration

The Bruker S2 Picofox TXRF equipment uses the S2 Picofox software to operate the TXRF spectrometer, adjust analytical parameters, and export results. The software generates both a spectrum graph and numerical quantifications of the counts under the curve for the detected elements, in terms of net area and concentration. To account for the possible presence of the target elements in the blank solution, the net area under the characteristic emission peak of each target element was used to calculate the element's concentration.

For every sample, a blank sample containing only 10 µL of yttrium standard and 5 mL of 1 % Triton X-100 solution was analysed, using the same analytical parameters. The representative spectra of the blank sample and the PRM-2002 sample scans are shown in Figure 2.

In the zoomed-in image, a slight peak near 10 keV is visible in the PRM-2002 spectrum. This peak corresponds to the characteristic emission line of Hg at 9.989 keV [20]. The S2 Picofox software automatically generates the net area values for each detected element after analysis. The net area values for both the blank and the PRM-2002 samples were recorded to calculate Hg concentrations. The Hg net area obtained from the blank

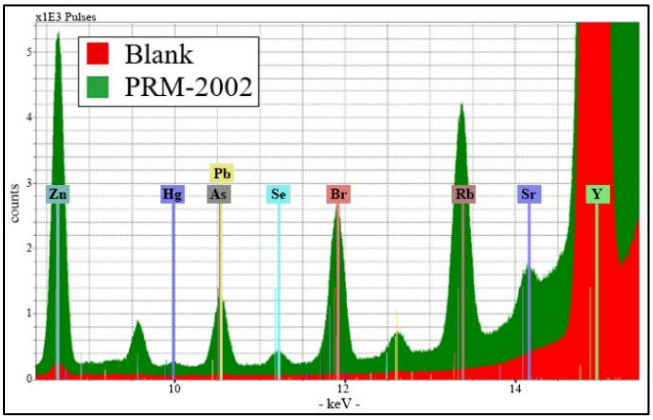

Figure 2. TXRF spectra containing a blank sample and a PRM-2002 sample scan zoomed in to observe the target element, Hg.

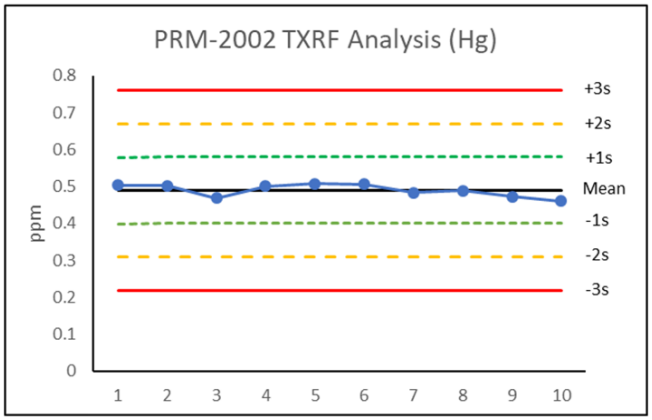

Figure 3. Levey–Jennings chart for TXRF analysis results of Hg in PRM-2002 samples.

samples was then subtracted from the Hg net area of the corresponding PRM-2002 samples before the concentration calculations were performed.

Equation (1) is used to calculate the concentration of the target heavy metal (C_{Hg}) using the following values reported by the TXRF:

A_{Hg} = net area of the heavy metal
S_{Hg} = sensitivity factor of Hg
C_Y = concentration of y std
A_Y = net area of y std
S_Y = sensitivity factor of y std

$$C_{Hg} = (C_Y \cdot A_{Hg} \cdot S_Y) / (A_Y \cdot S_{Hg}) . \quad (1)$$

Table 2 shows the calculated average Hg concentrations expressed in ppm and total recovery percentages. The Hg concentrations were calculated using equation (1) for the net area obtained from the TXRF analysis. The calculated overall average was 0.489 ppm. While the recovery percentages are within the 80–110 % range, which is the acceptable mean recovery for amounts between 100 ppm and 100 ppb [21]. It also shows that there are variations in Hg recovery, which could be due to noise signals during TXRF analysis, sample particle size, homogeneity of the sample suspension, thickness of the deposited sample on the quartz discs, and matrix effects.

To account for errors during the analysis, the Westgard rules were implemented for quality control. This method monitors possible significant deviations of data points or runs and quickly evaluates the accuracy and precision of the collected data. Figure 3 shows ten (10) data points plotted in the Levey–Jennings chart. There are several Westgard rules, e.g. a run is rejected if the measurement exceeds +/- 3 s (s = standard deviation), runs are ejected if two (2) consecutive runs exceed mean +/- 2 s, reject when four (4) consecutive runs exceed either mean +1 s or -1 s, reject if at least six (6) consecutive runs fall on one side of the mean, etc. The violation of the rules increases suspicion of the measurement method's accuracy and precision. Ultimately, the violations could lead to the analytical runs being deemed unacceptable [22]. As shown in Figure 3, the data points did not exceed the ± 1 s range, and no six consecutive runs fell on one side of the mean, which indicates that the runs are acceptable and the method has good precision in detecting Hg in milkfish samples, even at low concentrations.

Table 2. Calculated concentrations of Hg in multiple runs of PRM-2002 samples.

PRM Sample	Hg (ppm)	Hg Recovery (%)
1	0.503	102.653
2	0.502	102.449
3	0.468	95.510
4	0.500	102.041
5	0.507	103.469
6	0.506	103.265
7	0.483	98.571
8	0.489	99.796
9	0.473	96.531
10	0.460	93.878
Average	0.489	99.820

3.2. Relative standard deviation

The relative standard deviation (RSD), or coefficient of variation (CV), of the TXRF analysis for Hg in the PRM-2002 samples is also calculated (equation 2) to evaluate the precision of the method using PRM-2002:

$$RSD = \frac{sd}{\bar{x}} \cdot 100\% , \quad (2)$$

where sd = Standard deviation and $\bar{x}$ = Mean.

The RSD is 3.52 %, which indicates that the collected dataset is relatively precise and consistent. This also suggests that TXRF is capable of analysing Hg in milkfish samples. Therefore, it is suitable for detecting and initially evaluating Hg contamination in milkfish samples.

3.3. Measurement uncertainty

The Nordtest method was used to estimate the measurement uncertainty, specifically the technique that uses a single CRM. This method combines the uncertainty due to random effects, $u_{(Rw)}$, and the uncertainty due to bias, $u_{(bias)}$, to calculate the standard uncertainty, u_C [23]. Table 3 summarizes the calculated uncertainty components and the standard uncertainty.

Using the Nordtest method, the calculated expanded uncertainty was 0.49 ± 0.08 ppm.

Table 3. Nordtest method-calculated uncertainty components ($u_{(Rw)}$ and $u_{(bias)}$), standard uncertainty (u_C), and expanded uncertainty (U) in ppm.

$u_{(Rw)}$	$u_{(bias)}$	u_C	U
0.017	0.039	0.042	0.083

Table 4. Average calculated concentration and % recovery of Hg in PRM-2002 and DORM-5.

Sample	Hg (ppm)	% recovery
PRM-2002	0.489	99.820
DORM-5 (TXRF)	0.312	98.885

3.4. Analysis of milkfish samples

The milkfish samples were analysed using the optimized method. Six (6) adult-sized milkfish samples were collected from the sampling sites. Each milkfish was processed and analysed in triplicate, yielding a total of 72 data points (excluding blank samples). The net area values obtained from the analyses were recorded and used to calculate the concentration of Hg. In none of the six milkfish samples collected from five Laguna de Bay areas, an Hg concentration exceeding the detection limit of 0.06 ppm could be found.

The result shows that the amount of Hg in the dorsal meat of the collected milkfish samples is below the detection limit of the TXRF, which is 0.06 ppm. This is much smaller than the maximum limit of concern of 0.5 ppm [24]. As reference materials for Hg detection, PRM-2002 and DORM-5 samples were also analysed, as summarized in Table 4. The high recovery percentage indicates that the method had high accuracy during the sample analyses.

This generally indicates that the milkfish harvested in Laguna de Bay are well below toxic Hg levels. Given that multiple factors affect the bioaccumulation of mercury, and other heavy metals in general, the screening of milkfish samples only assesses consumption safety. The case may vary across different samples in the lake, such as other aquatic organisms, sediment, and lake water.

Figure 4 shows the spectra of a milkfish sample analysis overlaying the spectra of PRM-2002. The spectra show that there are slight peaks on the PRM-2002 at the Hg emission line, which the TXRF detected as Hg presence. In contrast, the milkfish sample spectrum does not have a visible peak in the Hg emission line, resulting in a null reading.

4. CONCLUSION

In conclusion, the study demonstrated that TXRF analysis is a reliable method for detecting Hg content in milkfish samples, even at low concentrations. The TXRF analysis of PRM-2002 samples yielded a 99.82 % recovery, which shows its accuracy for the analysis.

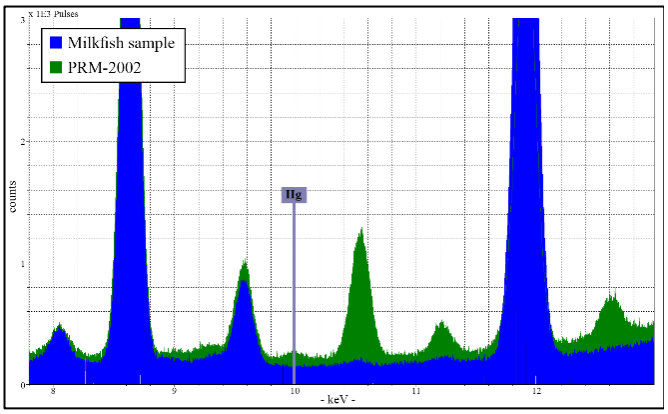

Figure 4. TXRF spectra of a milkfish sample and PRM-2002.

The method was used to screen Philippine milkfish (*Chanos chanos*) samples harvested from different areas around Laguna de Bay. The analyses show that the mercury (Hg) levels in the meat samples are well below 0.5 ppm, the limit of concern. This indicates that the milkfish harvested in Laguna de Bay are free from harmful Hg contamination and are safe for consumption.

ACKNOWLEDGEMENT

This work was supported by the Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) Grants-in-Aid [Grant number 021011012022-11-003198].

REFERENCES

[1] L. S. Meyor, P. J. S. Sajo, Obedencio Jr., J. M., V. Bautista-Jimenez, Mercury detection and histopathology of selected fish species in Jasaan Municipal Fishport, Misamis Oriental, The Philippine Journal of Fisheries 32 (2025) 2, pp. 365–377. DOI: 10.31398/tpjf/32.2.2024-0116

[2] Bureau of Fisheries and Aquatic Resources, 2023 Philippine Fisheries Profile (Annual country fisheries profile publication). Department of Agriculture, Republic of the Philippines (2025). Online [Accessed 5 January 2026] https://www.bfar.da.gov.ph/wp-content/uploads/2025/02/2023-Philippine-Fisheries-Profile.pdf

[3] W. Yap, A. C. Villaluz, M. G. Soriano, M. N. Santos, Milkfish production and processing technologies in the Philippines. Milkfish Project Publication Series No. 2 (2007).

[4] R. D. Lasco, E. Javier, Laguna de Bay: A Case Study for Sustainable Fisheries Development, Transactions of the National Academy of Science and Technology, 39 (2017), pp. 1-4. DOI: 10.57043/transnastphl.2017.1060

[5] E. D. Samson, The milkfish industry in the Philippines, Proc. of the Second International Milkfish Aquaculture Conference, Iloilo City, Philippines, 4–8 October 1983, pp. 215–228. Online [Accessed 10 January 2026] https://repository.seafdec.org.ph/handle/10862/163

[6] J. E. Fergusson, The heavy elements: Chemistry, environmental impact and health effects. Pergamon Press (1991).

[7] P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, D. J. Sutton, Heavy metal toxicity and the environment. Experientia Supplementum (2012) pp. 133–164. DOI: 10.1007/978-3-7643-8340-4_6

[8] World Health Organization: WHO, Mercury, 2024. Online [Accessed 14 August 2025] https://www.who.int/news-room/fact-sheets/detail/mercury-and-health

[9] D. van Rooyen, J. Erasmus, R. Gerber, M. Nachev, B. Sures, V. Wepener, N. J. Smit, Bioaccumulation and trophic transfer of total mercury through the aquatic food webs of an African sub-tropical wetland system, Science of the Total Environment 889 (2023), 164210. DOI: 10.1016/j.scitotenv.2023.164210

[10] K. Nyholt, T. D. Jardine, F. Villamarín, C. M. Jacobi, J. E. Hawes, J. V. Campos-Silva, S. Srayko, W. E. Magnusson, High rates of mercury biomagnification in fish from Amazonian floodplain-lake food webs, Science of the Total Environment 833 (2022), 155161. DOI: 10.1016/j.scitotenv.2022.155161

[11] T. Murphy, S. Lim, S. P. Huong, K. Irvine, S. Bayen, B. C. Kelly, K. Wilson, Application of handheld X-ray fluorescence analyzers to identify mercury in skin-whitening creams in Cambodia. Journal of Health and Pollution 2 (2012) 3, pp. 21–31. DOI: 10.5696/jhp.v2i3.32

[12] J. Q. McComb, C. Rogers, F. X. Han, P. B. Tchounwou, 2014. Rapid screening of heavy metals and trace elements in environmental samples using Portable X-Ray Fluorescence

Spectrometer, a comparative study, Water, Air, & Soil Pollution 225 (2014), 2169.
DOI: 10.1007/s11270-014-2169-5

[13] Y. Koleleni, O. Haji, Determination of concentration of heavy metals in fish from sea port of Zanzibar by energy dispersive X-ray fluorescence (EDXRF), Tanzania Journal of Science 40 (2014), pp. 80–89. Online [Accessed 20 September 2025] https://www.researchgate.net/publication/301771020_DETERMINATION_OF_CONCENTRATION_OF_HEAVY_METALS_IN_FISH_FROM_SEA_PORT_OF_ZANZIBAR_BY_ENERGY_DISPERSIVE_X-RAY_FLUORESCENCE_EDXRF

[14] P. Wobrauschek, Total reflection X-ray fluorescence analysis—a review, X-Ray Spectrometry, 36 (2007), pp. 289–300.
DOI: 10.1002/xrs.985

[15] A. Moya-Riffo, L. Bennun, V. Sanhueza, M. Santibañez, A procedure for overlapping deconvolution and the determination of its confidence interval for arsenic and lead signals in TXRF spectral analysis, X-Ray Spectrometry 42 (2013), pp. 93–99.
DOI: 10.1002/xrs.2439

[16] B. G. Beltrán, I. Martínez-Serrano, V. Ramos-Sanchez, D. Chávez-Flores, M. C. Nevárez-Rodríguez, E. A. Suárez-Domínguez, Development and validation of a new method for determination of PB and Cr in marine organisms by total reflection x-ray fluorescence (TXRF) spectroscopy, Journal of Analytical Methods in Chemistry (2019), pp. 1–7.
DOI: 10.1155/2019/8150678

[17] B. G. Beltrán, V. Ramos-Sanchez, D. Chávez-Flores, R. Rodríguez-Maese, E. Palacio, Total reflection X-ray fluorescence spectroscopy (TXRF) method validation: Determination of heavy metals in dietary supplements, Journal of Chemistry (2020), pp. 1–9.
DOI: 10.1155/2020/8817393

[18] P. A. Reis, L. M. P. Valente, C. M. R. Almeida, A fast and simple methodology for determination of yttrium as an inert marker in digestibility studies, Food Chemistry 108 (2008) 3, pp. 1094–1098.
DOI: 10.1016/j.foodchem.2007.11.060

[19] I. D. S. Magsino, C. J. Gatchalian, J. O. Esplana, G. E. D. Dizon, C. D. Laurio, A. C. Dacuya, A. T. Junsay, Development of Matrix Certified Reference Material for Toxic Elements in Milkfish for Accurate Trace Element Analysis, Measurement 265 (2026), 120386.
DOI: 10.1016/j.measurement.2026.120386

[20] A. Thompson, D. T. Attwood, E. M. Gullikson, M. R. Howells, K.-J. Kim, J. Kirz, (+9 more authors), X-ray Data Booklet. 3rd ed. Berkeley, CA: Lawrence Berkeley National Laboratory, 2009. Online [Accessed 17 September 2026] http://xdb.lbl.gov.

[21] I. Taverniers, E. Bockstaele, M. Loose, Analytical Method Validation and Quality Assurance. Pharmaceutical Manufacturing Handbook: Regulations and Quality (2010)
DOI: 10.1002/9780470571224.pse396

[22] J. O. Westgard, P. L. Barry, M. R. Hunt, T. Groth, A multi-rule Shewhart chart for quality control in clinical chemistry, Clin Chem. 27 (1981) 3, pp. 493–501. Online [Accessed 20 June 2025] https://westgard.com/downloads/papers-downloads/27-westgard-rules-paper/file.html

[23] B. Magnusson, T. Näykki, H. Hovind, M. Krysell, E. Sahlin, Handbook for calculation of measurement uncertainty in environmental laboratories (NT TR 537 - Edition 4) Denmark (2017).

[24] Bureau of Fisheries and Aquatic Resources. Fisheries Administrative Order No. 210-01: Rules and regulations on the exportation of fresh, chilled and frozen fish and fishery/aquatic products (FAO No. 210, s. 2001). Department of Agriculture, Republic of the Philippines, 2001, Online [Accessed 15 February 2025] https://www.bfar.da.gov.ph/wp-content/uploads/2021/04/FAO-No.-210-s.-2001.pdf