Clin. Lab. 2026;72:XXX-XXX

CASE REPORT

Unusually Elevated Triglycerides Due to Pure Water Contamination in Biochemical Analysis

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SUMMARY

Background: **Accurate measurement of serum triglycerides (TG) is essential for clinical diagnosis and treatment. Unexpectedly elevated TG levels in laboratory results may indicate analytical errors or contamination.**
Methods: **We report a case involving an unusual elevation of serum TG levels. Suspecting potential analytical interference, we first investigated issues related to quality control materials and reagents. To determine whether the pure water was the source of contamination, we performed manual TG testing. Five serum samples from the previous day, with TG concentrations of 0.72, 1.96, 2.93, 6.45, and 15.95 mmol/L, were selected for verification. A TG colorimetric strip was developed based on the final color of the enzymatic reaction system and used to compare pure water samples from the previous and current days.**
Results: **Quality control materials and reagents were ruled out as potential sources of interference for the unusually elevated TG levels. The TG reaction system for the current day's pure water produced a dark purple color, indicating a TG concentration theoretically exceeding 15.95 mmol/L. In contrast, the reaction system for the previous day's pure water showed a color comparable to the saline control. These findings confirmed the presence of TG contamination in the current day's pure water, which was ultimately traced to the recent replacement of the reverse osmosis membrane in the water purification system.**
Conclusions: **The manual TG detection method developed in this case offers a rapid and practical solution for laboratories with high automation to identify triglyceride contamination in pure water. This approach ensures the accuracy of biochemical test results and highlights the importance of monitoring pure water quality in laboratory settings.**
(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250605)

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KEYWORDS

pure water, triglyceride contamination, reverse osmosis membrane

INTRODUCTION

Pure water is an essential reagent in clinical laboratories [1], utilized for blanks, calibrators, controls, mobile phases, reaction mixtures, and reagent reconstitution [2]. In fully automated biochemistry analyzers, it also plays a critical role in cleaning laboratory components, such as sample and reagent needles, stirring rods, rinsing pools, cuvettes, and instrument pipelines. Consequently, the quality of pure water is vital for ensuring accurate test results.

Case Report accepted June 4, 2025

Currently, pure water in biochemical laboratories is primarily supplied by water purification systems. According to the People's Republic of China Health Industry Standard WS/T 574-2018, pure water must meet strict criteria: resistivity should be ≥ 10 MΩ·cm or a conductivity of ≤ 0.1 μS/cm (at 25℃), total organic carbon concentration < 500 ng/g (ppb), a total microbial count of < 10 CFU/mL, and fewer than one particle with a diameter of 0.22 μm or larger. When water quality does not meet these standards, it is necessary to replace components of the water purifier, such as the reverse osmosis (RO) membrane and ion exchange resin.

In this paper, we report a case of unusually elevated triglyceride (TG) levels resulting from pure water contamination caused by the replacement of the RO membrane.

CASE PRESENTATION

On September 5, 2024, the laboratory staff followed standard procedures to open the fully automated biochemical analyzer AU5831 and conducted a water blank test. TG concentrations, both high and low, were found to be out of control in subsequent quality control (QC) test results (Figure 1). The results exceeded the 3 standard deviation limits, coming in at 3.12 mmol/L and 5.26 mmol/L, respectively (the mean values of TG high and low concentration QC were 0.97 mmol/L and 1.26 mmol/L). QC results for all other parameters were within acceptable limits. Upon retesting with new QC materials, the TG results further increased to 4.18 mmol/L and 6.69 mmol/L.

Given the highly usual and unexpected QC results, potential causes such as reagent mix-up or contamination were suspected. After the reagents were replaced, QC was conducted again, and the results showed that the levels of 3.98 mmol/L and 6.46 mmol/L were still out of control. Following laboratory protocol for handling out-of-control results, TG calibration was carried out again, and it was observed that the calibration factor was significantly higher compared to previous data. Additionally, the reagent blank for TG showed a substantial increase. The reagents were confirmed to be within their validity period, and the manufacturer reported no quality problems with the batch. After excluding QC and reagent factors, the reason for the TG being out of control remains unclear, especially considering how inconsistently the deviation is measured each time.

Laboratory staff promptly organized a focused discussion to identify the cause. The laboratory has two fully automated biochemistry analyzers: Beckman Coulter AU5831 and AU5431. According to the laboratory daily workflow, AU5431 requires reagent addition every morning, resulting in a start time approximately one hour later than AU5831. During the discussion, the QC results for AU5431 were also obtained, showing that the TG results were similarly out of control, exceeding the 3SD limit. Both instruments exhibited uncontrolled and elevated TG levels, while QC results for all other parameters remained normal. This suggested that the issue might involve components shared by both instruments. The reagents, electricity, and water are the only parts that the two instruments have in common. Having already ruled out the reagent problem, focus shifted to other possible reasons. Other aspects would probably be impacted as well if electricity was the problem. Furthermore, based on our experience, electrical problems typically affect electrolyte measurements more, but the electrolyte QC results were within control. Therefore, the electrical supply was also ruled out as the cause.

This prompted us to investigate the water supply as a potential cause. We performed a manual TG test to find out if the contamination of pure water was due to uncontrolled TG levels. Our laboratory measures triglycerides using an enzymatic method that works as follows: triglycerides in the sample are hydrolyzed by lipoprotein lipase to form glycerol and fatty acids. Glycerol is then phosphorylated into 3-phosphoglycerol, which reacts with oxygen to produce dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2). Finally, H_2O_2 couples with the Trinder reaction to form a colored quinone imine, which results in an increase in absorbance that is directly proportional to the amount of TG concentration in the sample [3]. We selected remaining specimens with TG results of 0.72, 1.96, 2.93, 6.45, and 15.95 mmol/L, as measured the previous day, and used saline as a control for the manual TG assay. The samples were mixed with 30 μL of reagent 1 and 2,400 μL of saline, incubated at 37℃ for 5 minutes, and then 600 μL of reagent 2 was added, followed by an additional 5-minute incubation at 37℃. The colorimetric strip of TG was created by observing the color of the final reaction system (Figure 2), which was used to compare the pure water from the previous day and current day. The TG reaction system of the pure water from the current day was dark purple, suggesting a TG concentration theoretically surpassing 15.95 mmol/L, while the TG reaction system of the water from the previous day was comparable in color to the saline control (Figure 3). This finding validates that the unusual rise in TG levels was brought on by contamination of the pure water.

Subsequently, we immediately cut the water supply from the water purifier to the automated biochemistry analyzer, emptied the water purifier tank, and left the purifier to continue producing water until the TG color matched that of the saline control. We then let it produce water for an additional 20 minutes to rinse the tank thoroughly. Afterward, we successfully returned the QC to normal by reconnecting the instrument, performing the cleaning process twice, and recalibration of the water blank for TG.

This adverse laboratory event was resolved when the patients' samples tested normal. We think the contamination of the pure water occurred due to an engineer servicing the water purifier and replacing the RO membrane the night before. Usually, 180 g/L glycerin and 10 g/L $NaHSO_3$ are used to impregnate new, unopened

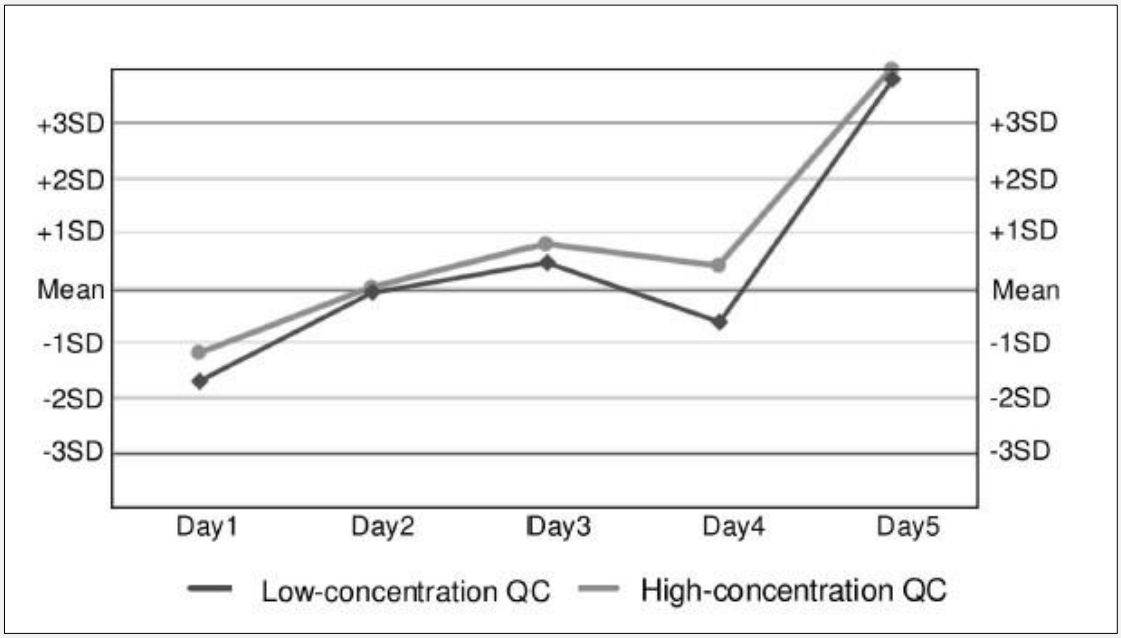

Figure 1. Quality control (QC) results for high and low triglyceride concentrations.

QC values remained within control limits during the first four days of September. On September 5th, both high and low QC concentration exceeded control limits by over 3 standard deviations (SD), indicating a loss of control.

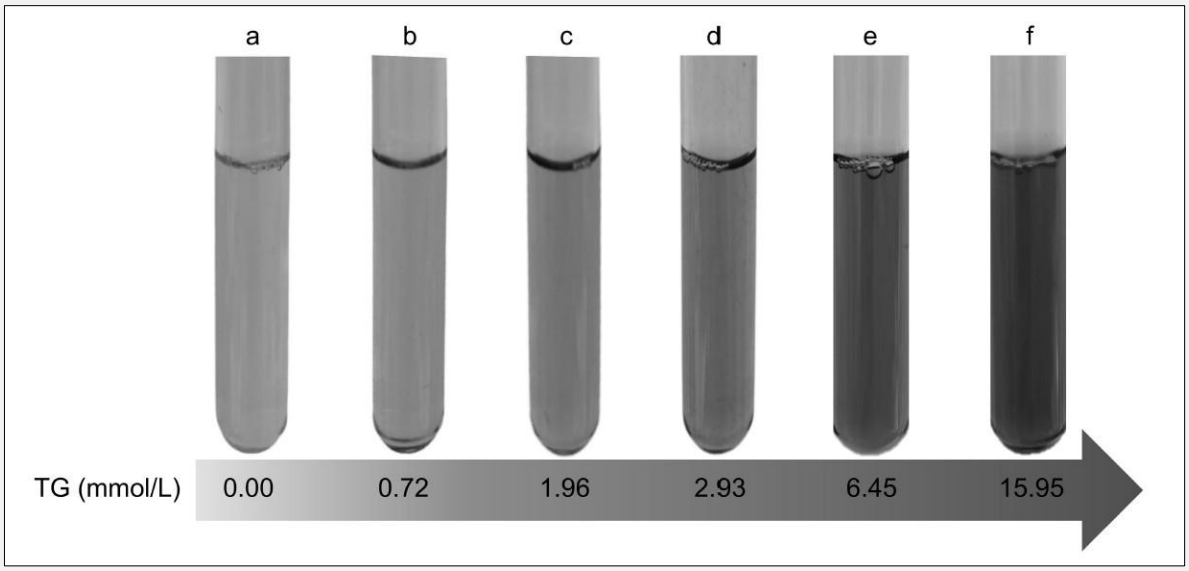

Figure 2. Homemade triglyceride colorimetric strips.

a Saline control, b - f Patient samples from the previous day.
Triglyceride assay results for each sample: b 0.72 mmol/L, c 1.96 mmol/L, d 2.93 mmol/L, e 6.45 mmol/L, and f 15.96 mmol/L.

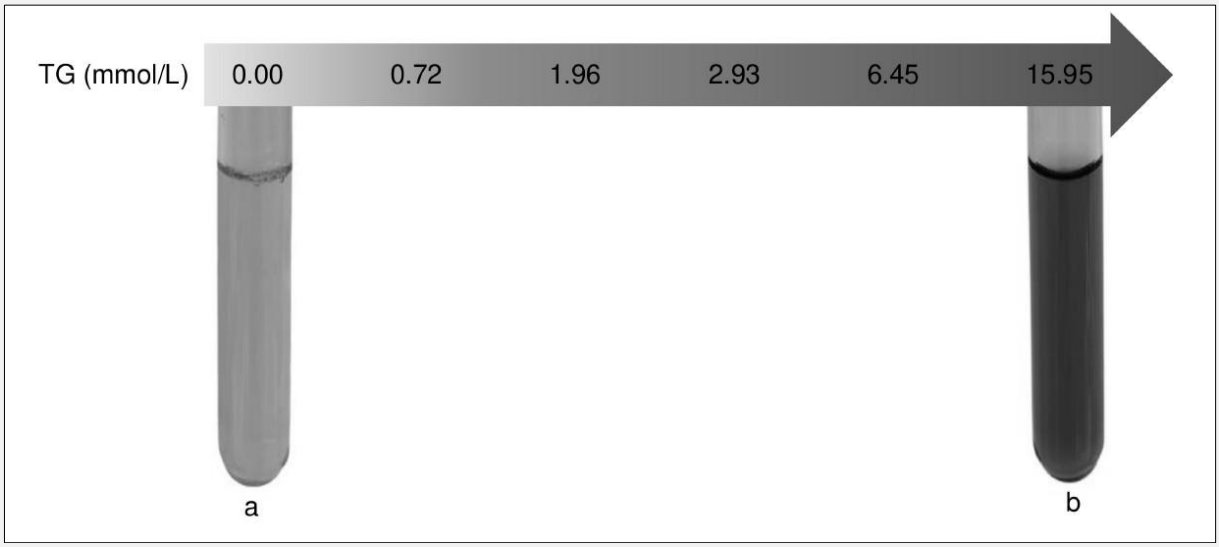

Figure 3. Comparison of TG reaction system between the previous day's pure water and the current day's pure water using the colorimetric strip.

a Previous day's pure water sample, b Current day's pure water sample. The experimental results indicate that the current day's pure water is contaminated with triglycerides.

RO membranes. To ensure that the membrane elements fit into the housing correctly during installation, engineers must lubricate the joints using glycerin or clean water. As a result, a certain amount of salt and glycerin are retained by the newly installed RO membrane. While the salt can be adsorbed by the ion exchange resin, glycerin, being a neutral molecule, is not adsorbed and, since it is fully water-soluble, can be carried into the biochemistry analyzer with the pure water. After replacing the RO membrane, engineers are required by standard procedure to rinse it thoroughly with pure water to get rid of the preservative solution and ensure the system is working properly before reconnecting it to the biochemistry analyzer. We believe that in this case, the engineer reconnected the water purifier to the analyzer without adequately rinsing the membrane, leading to contamination of the analyzer with glycerin residue.

DISCUSSION

Biochemical analyzers are essential instruments in clinical laboratories, used to analyze blood and other body fluids for various biochemical indicators. They assist in diagnosing diseases, monitoring treatment effectiveness, and managing chronic conditions. Their accuracy and automation minimize human error and greatly enhance testing efficiency [4,5].

In this paper, we report a case of falsely elevated triglyceride test results on a biochemical analyzer encountered in our daily work. We successfully confirmed our suspicion of triglyceride contamination in the pure water using a simple and rapid manual method: comparing the final color of the pure water reaction system before and after two days with handmade triglyceride colorimetric strips. In clinical practice, when facing similar issues that require immediate resolution, simple and intuitive color-based qualitative tests can be used without precise quantification. This approach enables rapid problem-solving, allowing timely issuance of laboratory reports and minimizing disruptions to daily workflow. Additionally, the daily reagent blank procedure was performed on the biochemistry analyzer after start-up, as described in this case; this effectively prevents errors in patient results due to reagent contamination. A reagent blank refers to background interference and contamination due to the test reagent itself, which is measured by routinely setting the amount of reagent and sample volume for the test and replacing the sample with pure water [6]. Therefore, reagent blanks can serve as an early warning signal to detect reagent contamination problems, including pure water, that would be difficult to identify. The routine implementation of daily reagent blank testing is not only an important means of ensuring reagent quality, but also a fundamental part of ensuring the reliability of the entire biochemical analysis process.

Ensuring pure water quality is essential for the reproducibility of biochemical experiments and the reliability of data. Primary sources of contamination in substandard pure water include electrolytes, organic compounds, particulates, microorganisms, and dissolved gases. Elevated electrolyte levels can impact ion measurements and enzyme activity assays, whereas organic contaminants can cause identical compounds to read inaccurately high. Particulates can clog the analyzer's pipelines, while microbial contamination may interfere with enzyme reactions or cause blockages in the system. Additionally, dissolved gases can alter the reaction environment, influencing test results [7].

Currently, our biochemical laboratory employs a combination of the RO membrane and electro-deionization technologies to produce pure water. The RO membrane removes salts, colloids, microorganisms, organics, and other impurities that the pre-filter cartridge does not capture. Due to its very small pore size, however, salts can accumulate on the membrane over time, leading to fouling and ultimately affecting the quality of the pure water [8]. The lifespan of the RO membrane is generally limited to 3 - 5 years [9], during which regular maintenance and proper cleaning by engineers are essential. Following maintenance and replacement of components, engineers must verify the quality of pure water before it is supplied to the biochemical analyzer; otherwise, it may compromise test accuracy, mislead patient diagnosis and treatment, and potentially result in clinical adverse events.

Source of Support:
This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Declaration of Interest:
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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