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Original Research Article

Influence of Water Source on the Outcome of Purification of Reagent Water in Lagos Mainland, Lagos Nigeria

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Abstract

Distilled water is not supposed to contain > 5µg of chlorides and if it does, distillation is unsuccessful, and a repeat is needed. The quantification of chlorides post purification of reagent grade water though serves to illustrate the success of purification, has also been used in this study to illustrate nearness to success. A total of 50 registered Clinical Chemistry Laboratories in Lagos Mainland of Lagos Nigeria were recruited in this cross sectional study, their reagent grade water sampled and tested for chlorides using spectrophotometric technique. A questionnaire was also administered to ascertain the Laboratories' source of water. In this study, significant amount of chloride was detected in all reagent water tested with concentration range of 0.055mg/L - 38.760mg/L. Most laboratories studied had Borehole as their source of water with few using bottled table water. The concentration of chlorides in laboratories using bottled table water ranged from 0.142 to 4.70. The average chloride concentration was 1.560mg/L and Variance of 2.953. This when compared to borehole sources with average chloride concentration of 5.912mg/L and variance of 79.378, at 95% confidence interval, p = 0.05, critical value 2.021 and calculated t = 2.896, was statistically significant. There is detection of significant concentrations of chloride implying failure of purification of water in all Laboratories studied. However, based on this study use of bottled table water is presently better than use of borehole as source of water for purification.
Keywords: Influence, water source, outcome, purification, Lagos, reagent water.

INTRODUCTION

Purified water constitutes the major component of many reagents, buffers, and diluents used in clinical laboratory testing. It can also become an indirect component of tests when it is used for washing and sanitizing instruments and laboratory ware, generating autoclave steam, etc. Purified water is a potential cause of laboratory error [1].

Evidence of errors introduced by poor grade water in clinical laboratories with particular reference to Clinical Chemistry Laboratory abound. Out of all impurities, the role of Chlorides has been documented for clinical laboratories as source of error [2].

On distillation, chlorine is volatilized and both chlorine and hypochlorous acid could appear in the distillate in appreciable quantities. In earlier studies, Chloride determinations by direct precipitation with mercuric nitrate were not affected by the small amount of chloride present in the chlorine contaminated water [3].

Distilled water is not supposed to contain > 5µg of chlorides and if it does, distillation is unsuccessful, and a repeat is needed.

The World Health Organisation (WHO) instructs that after preparation of distilled water, 1ml of 1.7% solution of silver nitrate should be added to 10ml of the distillate with addition of 2 drops of Nitric acid to check for the absence of chloride compounds (e.g. calcium chloride). The water should remain perfectly clear. If a slight whitish turbidity appears, the distillation process should be repeated [4].

The above instruction by WHO is the basis of this study, which enquires into the compliance with this and other similar standards by Clinical Chemistry Laboratories in our locality.

Water is the most frequently used reagent in the laboratory. Because tap water is unsuitable for laboratory applications, most procedures, including reagent and standard preparation, use water that has been substantially purified [5].

Preparation of many reagents and solutions used in the clinical laboratory requires "pure" water. Single-distilled water fails to meet the specifications for Clinical Laboratory Reagent Water (CLRW) established by the Clinical Laboratory and Standards Institute (CLSI) [6].

Distillation, ion exchange, reverse osmosis, and ultraviolet oxidation are processes used to prepare reagent grade water. In practice, water is filtered before any of these processes are used.(Bishop et al., 2005) A long-held convention for categorizing water purity was based on three types, I through III, with type I water having the most stringent requirements and generally suitable for routine laboratory use [7].

Traditionally, type II water was acceptable for most analytic requirements, including reagent, quality control, and standard preparation, while type I water was used for test methods requiring minimum interference, such as trace metal, iron, and enzyme analyses [7].

A number of application specific clinical laboratory methods require Type II Reagent Grade Water. The minimum quality for Reagent Grade Water (RGW) is indicated in the actual published test methods. The Clinical and Laboratory Standards Institute (formerly NCCLS) now govern these methods. Type II ASTM Reagent Grade Water quality exceeds the previously published NCCLS Type II laboratory water specification. Most laboratory environmental chambers, autoclaves, dishwashers, and humidifiers recommend the use of Type II RGW. Type II water does not contain minerals that will form scale in heating equipment or on glassware and should not leave residue after evaporation. Type II water is less aggressive towards wetted plumbing parts, pumps, and metal parts as compared to Type I water [8].

The distillate water must have a conductivity of less than 1.0 µS/cm (>1.0 MΩ·cm) at 25°C to meet Type II reagent grade water requirements. In addition, the distillate water must have a maximum TOC µg/L of 50 µg/L, maximum sodium of 5µg/L, maximum chloride of 5 µg/L, and maximum total silica of 3µg/L. Heterotrophic Bacteria Count (HBC) cfu/mL and bacterial endotoxin EU/mL level requirements vary upon Type II Grade requirements. [7]

The implication is that the presence of > 5 µg/L of chloride in laboratory reagent water disqualifies such water as a Type II RGW.

In reality, the laboratory reagent grade water system is the most important "instrument" in the lab. Reagent grade water quality will affect the precision and accuracy of every other instrument or test performed in the lab [2].

The Medical Laboratory Science Council of Nigeria (MLSCN), also in her guidelines for setting up secondary medical laboratories wherein bilirubin and other analytes affected by chlorides in RGW can be assayed, recommended distilled water for secondary medical laboratories [9].

However, there is no recommendation on the criteria such distilled water must meet as obtained in that of The American Society for Testing and Materials, known as ASTM International, which is a voluntary standards organization who publishes standards and specifications of quality for a multitude of materials as stated above. The MLSCN did not state the approach to ensuring reagent water grade used by clinical laboratories nor steps towards ensuring adherence at the long run but stipulates the sighting of Water Distiller as a criterion for accreditation of secondary clinical laboratories. Efforts at getting the guidelines of Medical and Dental Council of Nigeria on establishment of clinical laboratories in Nigeria, if any exists, proved abortive. It then follows that there are no stringent laws on Reagent Grade Water for Clinical Laboratories in Nigeria, leading to non-existence of unified terms of quality in Laboratory RGW.

From the above, it is obvious that lack of proper emphasis on RGW is tantamount to lack of full emphasis on quality assurance by these regulatory agencies.

MATERIALS AND METHODS

A total of 50 laboratories were included in the study.

Sampling method

Systematic using alphabetical order of names of Laboratories included.

Inclusion criteria

The inclusion criteria included:

- A facility must be a functional Clinical Chemistry Laboratory
- A facility must be assaying bilirubin in addition to other services
- The facility must be within Lagos Mainland in Lagos State
- The facility management must give informed consent and fill Study questionnaire

Exclusion criteria

Facilities were excluded because:

- They failed the inclusion criteria as listed above
- The managers opted out of the study

Testing laboratory water for chlorides

Materials and reagents

The materials needed and used include:

- Electronic weighing scale with lower and upper detection limits accommodating 1g to 10g respectively.
- Conical flasks (Erlenmeyer flask)
- Pipettes with elongated tips
- Measuring cylinder
- Standard flasks
- Beakers
- Funnels
- Weighing pan
- Glass rod for stirring
- 2.5L Brown bottle
- Stop watch or clock
- White filter paper
- Universal sterile bottles for collection of water samples from various facilities under study
- Register and writing material
- Spectrophotometer
- Cuvettes
- Test tubes and rack
- Micropipettes (50uL, 100uL and 1000uL) and micropipette tips

The chemicals needed and used include:

- Silver Nitrate ($AgNO_3$)
- Nitric acid (HNO_3)
- Reagent grade water from ISO 15189 certified laboratory (Pathcare South Africa)
- Commercially distilled water
- 0.9% normal saline

Sample handling and preservation

If sample is to be analysed within two hours of collection, there is no need for cooling but if beyond two hours, cool in a fridge but do not freeze sample.

Precautions to be taken

- AgNO3 must be stored in brown amber bottle and never exposed to sunlight
- Ensure AgNO3 does not spill on skin
- If AgNO3 spills on skin, the lesion caused could last for 10 to 15 days
- Ensure adequate lighting during observation for cloudy precipitates
- Ensure proper protocols at preparations of 1.7% silver Nitrate

Principle of method:

$$AgNO_3 + \quad XCl \xrightarrow{HNO_3} AgCl\,(ppt) \quad + \quad XNO_3$$

Silver nitrate will react and precipitate Chloride in solution with the cloudy precipitate observed as evidence of chloride in the distilled water. The Nitric acid as a donor of NO_3 drives the reaction forward and prevents reversibility.

Preparation of reagent

See appendix II

Procedure

This is a novel procedure for the detection and quantification of chloride in reagent grade water designed by this researcher based on the above recommendations by the World Health Organisation.

Spectral analysis

- 0.9% NaCl was diluted to 1:100.
- 100uL of 1.7% $AgNO_3$ was added to 1000uL of the diluted 0.9% NaCl (maintaining the 1:10 ratio as directed by WHO). 100uL of 0.1HNO3 was added to the mixture.
- The above was mixed and allowed to stand in the test tube at room temperature for 5 minutes.
- Spectral analysis was performed on the precipitate formed with 500nm discovered as the ƛ of maximum absorbance.

Callibrator or standard

- The 0.9% NaCl diluted to 1:100 was calculated to have 54.6mg/L of chlorine.
- 0.9% NaCl diluted to 1:100 was used as the standard in sample assay.

Controls

- Control 1 = Deionised
- Control 2 = Distilled water from ISO 15189 accredited laboratory
- Control 3 = 0.9% NaCl

Table for Assay Procedure

Test tubes	Blank	Standard	Sample
Water sample			1ml
0.9% NaCl diluted to 1:1000		1ml	
1.7% $AgN0_3$		100uL	100uL
$0.1HN0_3$		100uL	100uL
Mix and allow standing for 5 minutes at room temperature. Then read absorbance at 500nm. Concentration of chloride = $\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$ X 5.46mg/L			

Interpretation of results

- A zero absorbance = Nil Chloride detected
- A positive absorbance = Chloride detected
- Quantification of Chloride = Concentration of Chloride calculated

Record all findings.

Ethical Approval: From College of Medicine University of Lagos Health Research Ethical Committee.

RESULTS

Analytical Findings cum Questionaire Analysis

S/No	Sample concentration in mg/L	Location of Laboratory	Source of Water	Method of Purification
1	3.625	Surulere	Bore-hole	Distillation
2	21.998	Surulere	Bore-hole	Distillation
3	10.243	Mushin	Bore-hole	Distillation
4	1.496	Surulere	Bottled H_2O	UAP*
5	0.180	Surulere	Bore-hole	Distillation
6	38.760	Mushin	Bore-hole	Distillation
7	1.911	Surulere	Bore-hole	Distillation
8	2.271	Surulere	Bore-hole	Distillation
9	22.517	Isolo	Bore-hole	Distillation
10	6.219	Surulere	Bore-hole	Distillation
11	3.249	Isolo	Bottled H_2O	UAP
12	3.429	Surulere	Bore-hole	Distillation
13	4.286	Surulere	Bore-hole	Distillation
14	1.496	Surulere	Bore-hole	Distillation
15	4.423	Surulere	Bore-hole	Distillation
16	0.683	Surulere	Bore-hole	Distillation
17	5.220	Isolo	Bore-hole	Distillation
18	2.888	Isolo	Bore-hole	Distillation
19	7.273	Oshodi	Bore-hole	Distillation
20	3.707	Isolo	Bore-hole	Distillation
21	4.144	Mushin	Bore-hole	Distillation
22	2.091	Ikeja	Bore-hole	Distillation
23	2.708	Mushin	Bore-hole	Distillation
24	0.099	Ikeja	Eva water	UAP
25	2.233	Ikeja	Bore-hole	Distillation
26	4.701	Isolo	Bottled H_2O	UAP
27	0.300	Isolo	Eva water	UAP
28	0.055	Surulere	Bore-hole	Distillation
29	19.607	Surulere	Bore-hole	Distillation
30	6.295	Mushin	Bore-hole	Distillation
31	0.142	Surulere	Bottled H_2O	UAP
32	0.180	Surulere	Bore-hole	Distillation
33	30.571	Mushin	Bore-hole	Distillation
34	0.060	Surulere	Bore-hole	Distillation
35	0.240	Surulere	Bore-hole	Distillation
36	20.819	Isolo	Bore-hole	Distillation
37	4.106	Surulere	Bore-hole	Distillation
38	0.142	Isolo	Bore-hole	Distillation
39	0.142	Surulere	Bore-hole	Distillation

40	0.595	Surulere	Bore-hole	Distillation
41	0.158	Surulere	Bore-hole	Distillation
42	1.037	Surulere	Bore-hole	Distillation
43	0.120	Surulere	Bore-hole	IER+
44	7.114	Isolo	Bore-hole	Distillation
45	0.437	Oshodi	Bore-hole	Distillation
46	0.480	Isolo	Bore-hole	Distillation
47	0.240	Mushin	Purchased	UAP
48	3.686	Mushin	Bore-hole	Distillation
49	0.158	Mushin	Bore-hole	Distillation
50	2.250	Mushin	Bottled H_2O	UAP

UAP* = Used as Purchased, IER+ = Distillation + Ion Exchange Resin

Discussion

The presence of > 5 µg/L of chloride in laboratory reagent water disqualifies such water as a Type II RGW [7].

Type II water is acceptable for most analytic requirements, including reagent, quality control, and standard preparation, while type I water was used for test methods requiring minimum interference, such as trace metal, iron, and enzyme analyses. Type II water does not contain minerals that will form scale in heating equipment or on glassware and should not leave residue after evaporation. Type II water is less aggressive towards wetted plumbing parts, pumps, and metal parts as compared to Type I water [8].

The above implies that the laboratory reagent grade water system is the most important "instrument" in the lab since grade water quality will affect the precision and accuracy of every other instrument or test performed in the laboratory [10].

In Nigeria the main sources of water which are bore-hole and pipe-borne are treated by heavy chlorination. Some distillation processes may also result in the inadvertent contamination of distilled water with free chlorine in most climes. One part per million of free chlorine in distilled water has been shown to inhibit markedly the colour development in the usual determinations of uric acid and bilirubin. The effect of chlorine on other clinical chemistry determinations like potassium is shown [11].

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Most laboratories studied had Borehole as their source of water with few using bottled table water. The concentration of chlorides in laboratories using bottled table water ranged from 0.142 to 4.701. The average chloride concentration was 1.560mg/L and Variance of 2.953. This when compared to borehole sources with average chloride concentration of 5.912mg/L and variance of 79.378, at 95% confidence interval, p = 0.05, critical value 2.021 and calculated t = 2.896, was statistically significant. This implies that the use of bottled table water is presently better than use of borehole distillate.

The failure in distillation could be attributed to the source of water. This is because borehole and pipe-borne water contain up to 250mg/L of chloride prior to purification making it unsuccessful [12, 13]. This is keeping with findings by other researchers that the source of water affects the outcome of purification of drinking water [14].

This could have been detected if there is culture of testing post purification. This study shows that 100% of laboratory studied do not test their purified water to assess quality. This malpractice of not testing water post purification is even worsened by long duration of storage of water post purification. The storage gallons are sparingly washed in all laboratories studied. During storage, contamination may occur whenever the container is breached or by leaching of minerals or compounds from the container into the distillate [15].

Conclusion

There is detection of significant concentrations of chloride implying failure of purification of water in all Laboratories studied. However, based on this study use of bottled table water is presently better than use of borehole as source of water for purification.

Recommendations

There is need for multiple repetitions of distillation processes especially due to very high concentrations of chloride in the initial water in our clime. There is need for Nigerian Laboratory regulatory agencies to come up with step by step guidelines on preparation and utilisation of reagent water.

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