ORIGINAL ARTICLE

USING DETECTION OF CHLORIDES AFTER PURIFICATION OF REAGENT WATER AS A MEASURE TO DETERMINE CONFORMITY WITH MINIMAL GRADE QUALITY IN LAGOS MAINLAND, LAGOS, NIGERIA

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ABSTRACT

Less than 5µg of chloride content must be achieved to adjudge water distillation successful. The World Health Organisation (WHO) instructs that post distillation of 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 detect chloride compounds (e.g. calcium chloride). The water should remain perfectly clear. If slightly cloudy, the distillation process should be repeated. This study enquires into the compliance; with this and other similar Standards, by Clinical Chemistry Laboratories in our locality. 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 using the above directives by WHO post informed consent. We used an accurate, sensitive and simple, novel methodology to do this.

In this study, there is detection of significant concentrations of chloride in RGW of 100% of Laboratories studied with concentration range of 0.055mg/L - 38.760mg/L. Using chloride concentration as a guide, there is failure of purification of water in all Laboratories studied.

KEY WORDS: Chloride, Water, Reagent water, Purification, Detection, Lagos, Nigeria, Laboratory, Error, Quality

INTRODUCTION

Water is commonly used reagent in the laboratory. Due to unsuitability of tap water for laboratory applications, most procedures, including reagent and standard preparation, use water that has been substantially purified.[1] “Pure” water is required for preparation of many reagents and solutions used in the clinical laboratory. Single-distilled water often fails to meet the specifications for Clinical Laboratory Reagent Water (CLRW) established by the Clinical Laboratory and Standards Institute (CLSI).[2] Distillations, ion exchange, reverse osmosis and ultraviolet oxidation singly or combined are processes used to prepare reagent grade water. In practice, water is filtered prior to any of these processes are used.[1] A long-held water purity classification was based on three types, I through III, with type I water having the most stringent requirements and generally suitable for routine laboratory use.[3]

Since life needs an answer, the role of diagnostic medicine in health and in disease cannot be over emphasised. This is because the healthy need to be reassured that based on available body chemistry indices, such healthy living and standard would not likely deteriorate, and the ailing need to be informed that the alteration in body chemistry that resulted in illness has been identified, with hope for correction elucidated.[4]

These information represented on a result sheet in a reply to an earlier request from a Clinician, is the boldness and conviction of the Clinician. His actions and information are dependent on this laboratory output. This means that words and acts that kill are dependent on it.[5]

A key element of success in the clinical laboratory is the consistency of test result quality. The physician or caregiver counts on results that represent only patient analytes measurement, not the measurement of microbial or chemical contaminants that may be extraneously introduced during a laboratory procedure.[6]

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.[7]

Errors introduced by poor grade water in clinical laboratories especially Clinical Chemistry Laboratory are established. Of all impurities, the role of chlorides has been demonstrated for clinical laboratories as source of error.[8]

Traditionally, type II water was acceptable and recommended 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.[3]

Most application specific clinical laboratory methods require Type II Reagent Grade Water. The minimum quality for Reagent Grade Water (RGW) needed is indicated in the actual published test methods. The Clinical and Laboratory Standards Institute (formerly NCCLS) presently govern these methods. Type II American Society for Testing and Materials (ASTM) Reagent Grade Water quality exceeds the earlier published NCCLS Type II laboratory water specification. A good number of laboratory environmental chambers, autoclaves, dishwashers, and humidifiers recommend the use of Type II RGW. Type II water is devoid of 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 and minimally damages wetted plumbing parts, pumps, and metal parts as compared to Type I water.[9]

Conductivity of less than 1.0 µS/cm (>1.0 MΩ·cm) at 25°C is a criteria to meet Type II reagent grade water requirements. Further criteria is that 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. The Heterotrophic Bacteria Count (HBC) cfu/mL and bacterial endotoxin EU/mL level requirements vary upon Type II Grade requirements.[3]

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

It is obvious that the laboratory reagent grade water system is the most important “instrument” in the laboratory since water quality will affect the precision and accuracy of every other instrument or test performed in the lab.[8] The Medical Laboratory Science Council Of Nigeria (MLSCN), also directs that in setting up secondary medical laboratories wherein bilirubin and other analytes affected by chlorides in RGW can be assayed, distilled water should be used.[10]

The council did not make recommendation on the criteria such distilled water must meet as obtained in that of The American Society for Testing and Materials (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 was silent on the approach to ensuring that reagent water grade is used by clinical laboratories. She did not outline steps towards ensuring adherence at the long run but stipulates the sighting of Water Distiller as a criterion for accreditation of secondary clinical laboratories. Guidelines of Medical and Dental Council of Nigeria on establishment of clinical laboratories in Nigeria could not be sourced. These imply 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.

Chlorine is volatile, and both chlorine and hypochlorous acid could appear in the distillate in appreciable quantities. Earlier studies, showed that Chloride determinations by direct precipitation with mercuric nitrate were not affected by the small amount of chloride present in the chlorine contaminated water.[11] Distilled water should not contain > 5µg of chlorides and if it does, distillation is unsuccessful, and a repeat is needed. The World Health Organisation (WHO) requests 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 brilliantly clear. An instance of a slight whitish turbidity implies that the distillation process should be repeated.[12] The basis of this study is the above instruction by WHO. The study enquires into the compliance with this and other similar standards by Clinical Chemistry Laboratories in our locality.

1. MATERIALS AND METHODS

A total of 50 laboratories were included in the study.

2.2 SAMPLING METHOD: Systematic using alphabetical order of names of Laboratories included.

2. 3 INCLUSION CRITERIA

The inclusion criteria included:

1. A functional Clinical Chemistry Laboratory
2. A facility must be assaying bilirubin in addition to other services
3. The facility must be within Lagos Mainland in Lagos State
4. The facility management must give informed consent and fill study questionnaire

2.4 EXCLUSION CRITERIA

Facilities were excluded because:

1. They failed the inclusion criteria as listed above
2. The managers opted out of the study

2.5 TESTING LABORATORY WATER FOR CHLORIDES

2.5.1 MATERIALS AND REAGENTS

The materials needed and used include:

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

2.5.1 THE CHEMICALS NEEDED AND USED INCLUDE

1. Silver Nitrate ($AgNO_3$)
2. Nitric acid (HNO_3)
3. Reagent grade water from ISO 15189 certified laboratory (Pathcare South Africa)

4. Commercially distilled water
5. 0.9% normal saline

2.5.2 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.

2.5.3 PRECAUTIONS TO BE TAKEN

1. $AgNO_3$ must be stored in brown amber bottle and never exposed to sunlight
2. Ensure $AgNO_3$ does not spill on skin
3. Ensure adequate lighting during observation for cloudy precipitates
4. Ensure proper protocols at preparations of 1.7% silver Nitrate

2.5.4 PRINCIPLE OF METHOD

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

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.

2.5.5 PREPARATION OF REAGENT

See appendix II

2.5.7 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.[12]

2.6 SPECTRAL ANALYSIS

1. 0.9% NaCl was diluted to 1:100.
2. 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 $0.1HNO_3$ was added to the mixture.
3. The above was mixed and allowed to stand in the test tube at room temperature for 5 minutes.
4. Spectral analysis was performed on the precipitate formed with 500nm discovered as the λ of maximum absorbance.

2.7 CALLIBRATOR OR STANDARD

1. The 0.9% NaCl diluted to 1:100 was calculated to have 54.6mg/L of chlorine.
2. 0.9% NaCl diluted to 1:100 was used as the standard in sample assay.

2.7.1 CONTROLS

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

TEST TUBES	BLANK(Air blanking)	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 = Absorbance of sample X 5.46mg/L Absorbance of standard			

2.9 INTERPRETATION OF RESULTS

1. A zero absorbance = Nil Chloride detected
2. A positive absorbance = Chloride detected
3. Quantification of Chloride = Concentration of Chloride calculated

3. 0 RESULTS

Results of Analysis

S/No	Abs sample 1	Abs sample 2	Ave. Abs sample	Abs of Std 1	Abs of Std 2	Average Abs of Std	Abs sample /Abs Std	Std Conc. (mg/L)	(Abs sample/Abs Std) x Std Conc	Sample conc in mg/L
1	0.181	0.183	0.182	0.273	0.275	0.274	0.664	5.46	3.625	3.625
2	1.103	1.105	1.104	‘’	‘’	‘’	4.029		21.998	21.998
3	0.513	0.515	0.514	‘’	‘’	‘’	1.876		10.243	10.243
4	0.078	0.072	0.075	‘’	‘’	‘’	0.274		1.496	1.496
5	0.010	0.008	0.009	‘’	‘’	‘’	0.033		0.180	0.180
6	1.946	1.944	1.945	‘’	‘’	‘’	7.099		38.760	38.760
7	0.097	0.095	0.096	‘’	‘’	‘’	0.350		1.911	1.911
8	0.110	0.118	0.114	‘’	‘’	‘’	0.416		2.271	2.271
9	1.140	1.120	1.130	‘’	‘’	‘’	4.124		22.517	22.517
10	0.313	0.311	0.312	‘’	‘’	‘’	1.139		6.219	6.219
11	0.165	0.161	0.163	‘’	‘’	‘’	0.595		3.249	3.249
12	0.174	0.170	0.172	‘’	‘’	‘’	0.628		3.429	3.429
13	0.217	0.213	0.215	‘’	‘’	‘’	0.785		4.286	4.286
14	0.079	0.071	0.075	‘’	‘’	‘’	0.274		1.496	1.496
15	0.224	0.220	0.222	‘’	‘’	‘’	0.810		4.423	4.423
16	0.037	0.033	0.035	‘’	‘’	‘’	0.128		0.683	0.683
17	0.263	0.261	0.262	‘’	‘’	‘’	0.956		5.220	5.220
18	0.150	0.140	0.145	‘’	‘’	‘’	0.529		2.888	2.888
19	0.370	0.360	0.365	‘’	‘’	‘’	1.332		7.273	7.273
20	0.191	0.181	0.186	‘’	‘’	‘’	0.679		3.707	3.707
21	0.035	0.021	0.028	‘’	‘’	‘’	0.759		4.144	4.144
22	0.106	0.104	0.105	‘’	‘’	‘’	0.383		2.091	2.091
23	0.137	0.135	0.136	‘’	‘’	‘’	0.496		2.708	2.708
24	0.006	0.004	0.005	‘’	‘’	‘’	0.018		0.099	0.099
25	0.114	0.110	0.112	‘’	‘’	‘’	0.409		2.233	2.233
26	0.237	0.235	0.236	‘’	‘’	‘’	0.861		4.701	4.701
27	0.016	0.014	0.015	‘’	‘’	‘’	0.055		0.300	0.300
28	0.002	0.004	0.003	‘’	‘’	‘’	0.010		0.055	0.055
29	0.895	0.893	0.984	‘’	‘’	‘’	3.591		19.607	19.607
30	0.317	0.315	0.316	‘’	‘’	‘’	1.153		6.295	6.295
31	0.008	0.006	0.007	‘’	‘’	‘’	0.026		0.142	0.142
32	0.010	0.008	0.009	‘’	‘’	‘’	0.033		0.180	0.180
33	1.535	1.533	1.534	‘’	‘’	‘’	5.599		30.571	30.571

34	0.004	0.002	0.003	‘’	‘’	‘’	0.011		0.060	0.060
35	0.011	0.013	0.012	‘’	‘’	‘’	0.044		0.240	0.240
36	1.050	1.040	1.045	‘’	‘’	‘’	3.813		20.819	20.819
37	0.205	0.207	0.206	‘’	‘’	‘’	0.752		4.106	4.106
38	0.008	0.006	0.007	‘’	‘’	‘’	0.026		0.142	0.142
39	0.006	0.008	0.007	‘’	‘’	‘’	0.026		0.142	0.142
40	0.040	0.020	0.030	‘’	‘’	‘’	0.109		0.595	0.595
41	0.007	0.009	0.008	‘’	‘’	‘’	0.029		0.158	0.158
42	0.051	0.053	0.052	‘’	‘’	‘’	0.190		1.037	1.037
43	0.005	0.007	0.006	‘’	‘’	‘’	0.022		0.120	0.120
44	0.358	0.356	0.357	‘’	‘’	‘’	1.303		7.114	7.114
45	0.021	0.023	0.022	‘’	‘’	‘’	0.080		0.437	0.437
46	0.028	0.020	0.024	‘’	‘’	‘’	0.088		0.480	0.480
47	0.011	0.013	0.012	‘’	‘’	‘’	0.044		0.240	0.240
48	0.190	0.180	0.185	‘’	‘’	‘’	0.675		3.686	3.686
49	0.009	0.007	0.008	‘’	‘’	‘’	0.029		0.158	0.158
50	0.114	0.112	0.113	‘’	‘’	‘’	0.412		2.250	2.250

Std = Standard, Conc. = Concentration, Abs = Absorbance

SUMMARY OF FINDINGS

PERCENTAGE OF LABORATORIES STUDIED	RANGE OF CHLORIDE CONCENTRATION	REMARKS
100%	0.055mg/L - 38.760mg/L.	Failed
86%	<10mg/L	Failed
76%	<5mg/L	Failed
36%	<1000µg/L	Failed
32%	<500µg/L	Failed
6%	<100µg/L	Failed

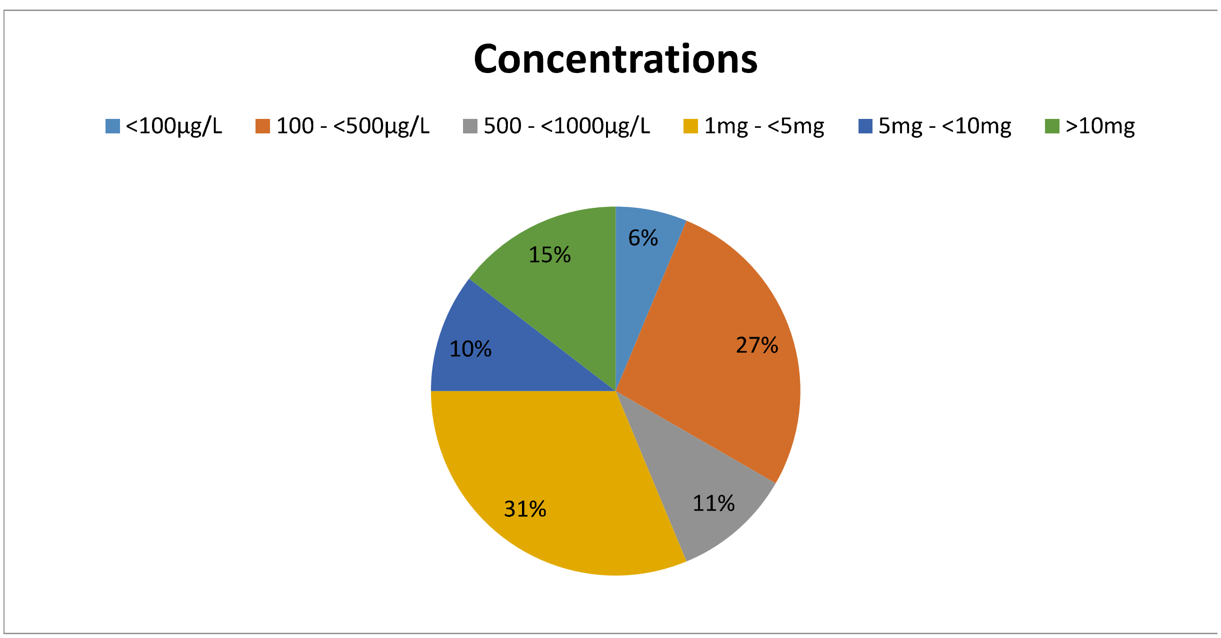

Figure 1: Pie Chart of chloride concentrations in the laboratories studied.

4.0 DISCUSSION

The laboratory reagent grade water system can be described as 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 lab.[13]

In Nigeria bore-hole and pipe-borne water are the main sources of water and are treated by heavy chlorination. In other climes, sea water may be their major source of water, while 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 demonstrated 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 demonstrated.[14]

The Medical Laboratory Science Council Of Nigeria (MLSCN), 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 and a distiller must be sighted during accreditation visits.[10]

MLSCN did not outline 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 stipulates the sighting of Water Distiller as a criterion for accreditation of secondary clinical laboratories but did not state the approach to ensuring that reagent grade water is used by clinical laboratories nor steps towards ensuring adherence at the long run.

This suggests 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. This implies a lack of full emphasis on quality assurance by these regulatory agencies.

In this study, significant amount of chloride was detected in all reagent water tested with concentration range of **0.055mg/L - 38.760mg/L.** This is about one-sixth the upper permissible level in drinking water.[15]

Six per cent (6**%)** of Laboratories studied have **<100µg/L of chlorides** in their purified reagent water, while **32%** have **<500µg/L, 36%** have **<1000µg/L, 76%** have **<5mg/L, 86% <10mg/L.** This is the first of this type of study in Nigeria.

4.0 CONCLUSION

There is detection of significant concentrations of chloride in RGW of 100% of Laboratories studied. Using chloride concentration as a guide, there is failure of purification of water in all Laboratories studied.

6.0 LIMITATION

Paucity of similar research works limiting comparing of methods and findings.

7.0 RECOMMENDATIONS

Laboratory Regulatory Bodies should institute steps at ensuring purification of Reagent Grade water and determining the success of the entire process.

There is need for more research into this aspect of Quality in Clinical Laboratory since there is paucity of works in this regard.

We used an accurate, sensitive and simple, novel methodology in this work. There is need for wide research on this method. The method will form a simple and reliable one for post purification test on purified reagent grade water for clinical laboratories especially in African and other poor resource countries.

8.0 ACKNOWLEDGEMENT

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