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INSTABILITY OF LABORATORY REAGENTS FACE TO ENVIRONNEMENTAL WORKING AND CLIMATE CHANGE IN THE EAST NORTHEN HOSPITAL IN BENIN

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ABSTRACT

Risks related to environment working and those related to climate change influences the stability of diagnostic reagents and therefore laboratories results quality in both departments of Borgou and Alibori. Reagents used in real conditions of use in two hospitals areas of Alibori and Borgou during three-month period covering harmattan period and extreme heat periods shown a relatively high degree of instability. Materials Dyasis used to perform calcium, transaminases and glucose tests control shown statistically significant differences and values are outside Levey Jennings confidence area. These values are beyond the limit of 10 unit's apart confidence for transaminase and 5 units for calcium showing that temperature, moisture action, light and contaminants changed physical and chemical equilibrium reagents state which may pass from static to dynamic. Only glucose reagent shown integrity relative face to hygroscopic conditions, thermal sensitivity and ambient contaminants.

Keywords: Diagnostic reagents instability, environment, climate effects, Alibori, Borgou, Benin

1- INTRODUCTION

Pharmaceutical and sanitary products must contribute to good healthy for men. Health products such as biomaterials, medical devices and in vitro diagnostic devices must require a good selection, rather strict storage conditions and strict compliance with data suppliers. The quality of these products and their integrity must be preserved. Pharmaceutical and sanitary products, before they reach the users must receive strict supervision from the development, manufacture, placing on the market [1]. Physical or chemical state of these products may pass from static to dynamic when hygroscopic effects, heat sensitivity, photosensitivity or evens contamination situations happened [2]. Extreme humidity and hot temperature may influence pharmaceutical and sanitary products. Also laboratory reagents are opened each time and may stay opened in laboratory conditions for a long time. Hot climatic conditions of Borgou and Alibori Department can easily show difficulties to maintain integrity of these products. Also, systemic risks associated with environment work in diagnostic laboratories, pharmaceutical management procedures are not sure to guarantee physicochemical stability of these products in these two departments. It seems appropriate to take an interest in the quality management process of these products for the maintenance of their integrity. It is therefore necessary to evaluate physical and chemical changes observed when climatic conditions, electric energy distribution disruptions, quality management practices and management standards quality of diagnostic reagents in laboratories through real life stability tests of transaminase and calcium reagents.

2- MATERIAL AND METHODS

This study were conducted over a period of three months (January, February and March) in Kandi and Malanville hospitals in the departement of Alibori and in Parakou and N 'dali hospitals in Borgou departement.

Study areas

2-1 Climatic characteristics of Borgou and Alibori

2-1-1 Borgou

Borgou is located in Guinea-Sudan area between 7°30'N and 9° 45'N extends from Dassa latitude to Bembèrèkè. Parakou and N'dali sanitary area is located in Borgou departement. It is characterized by the fusion of two rainfall peaks (unimodal) and marks a transition to a typical soudanese climate. The average annual rainfall varies from 900 mm to 1110 mm, mostly spread over an average of 113 days. Relative humidity ranged from 31% to 98% and temperatures ranged from 25 °C to 29 °C.

2-1-2 Alibori

Kandi and Malanville hospitals area are common to in the Alibori department and islocated in Soudan area (between 9° 45 'N and 12° 30' N). The climate is typically Soudanian and its rainfall varied from 900 to 1100 mm per year and were distributed on average over 145 days. The air humidity ranged from 18% during the harmattan (December to February) to 99% in August during the rainy season and the monthly average temperature ranged from 24°C to 31°C.

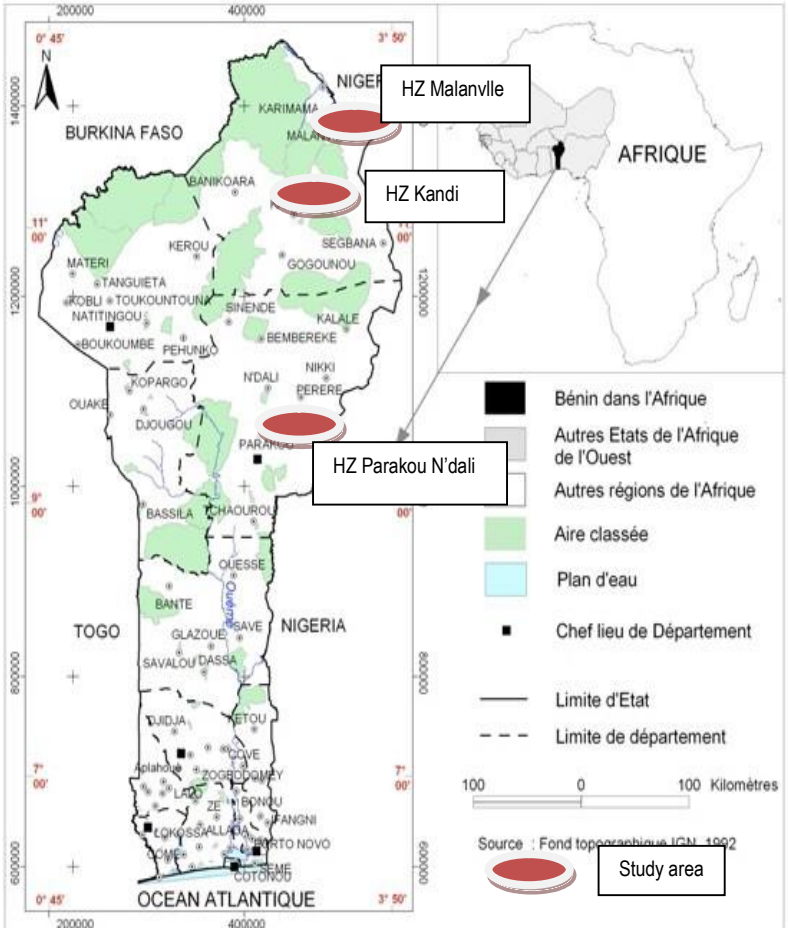

Figure 1: Sampling map

2-2 Sampling

Two hundred and forty (240) tests were carried out on just opened laboratories reagents and during reagents in use in hot temperature and harmattan period

2-3 Equipment

1- It is composed of two reagents (Chromatest and Biolabo) for performing transaminases, calcium and glucose tests.

2- An equipment control (biochemicalcon trol) of Dyasis variety manufactured by Dyasis Diagnostic System GmbH w ere used in laboratory to control and to test transaminases, calcium and glucose parameters.

3- Multiparameter spectrophotometer MI NDRAY BA 88A

4- Bacterial culture media such as Chapman and Mac Conckey were used to assess bacterial growth.

2-4 METHODOLOGY

Ten testing reagents and control serum (normal and pathological) were conducted to evaluate analytical process accuracy and precision. Lyophilisate serum is reconstituted according to the manufacturer's recommendations. In December, all purchasednormal and pathological reagents were reconstituted as tests recommendations and were tested with Dyasis TruLab for two variety reagents such as Chromatest and Biolabo for three parameters such as transaminase, calcium, and glucose.

The same reagents were sent to laboratories of Kandi, Malanville and Boko's hospital to be used according to the usual work conditions for three months. Coolers are used for transportation. Three months after, the remaining third of these reagents were returned to laboratory for stability valuation.

Analytical process validation and stability of transaminase calcium and glucose reagents just opened and in use during three months were done.

Statisticalanalysis were done by reassessment of:

- the accuracy by comparing the average values of tests to true value of the serum control with the Student t test at the α risk of 5% and ddl equal to 9 on the one hand, and 4 other units (transaminases);
- the accuracy is evaluated by calculating intraseriel and interseriel coefficients variation. This involves repeatability and reproducibility assessment.

Finally for the integrity of the study, bacterial growth on suitable culture are analyzed in relation to unnatural environments conditions.

3– RESULTS

3-1 Analytical process validation

Analytical methods used for the biochemical parameters measurement are validated by the manufacturers of the reagents used. Precision and accuracy of our analytical processes are evaluated with serum Dyasis control (normal and abnormal) and the reagents showed in Table 1 below.

Results obtained to confirm accuracy and precision of different methods and stability of the opened reagents used before sending to the areas are given in the following table 2.

Tests conducted with normal and pathological control serum shown that the average values for each parameter are close to the target average values of the control equipment. Student t test comparison shown that there is no significant difference between the real values of the control equipment and our average values obtained for α risk of 5%. Intraseriel and interseriel variation coefficients were lower than 2%. This

confirms the accuracy of the methods and the reagents stability when opened.

3-2 TESTS ON REAGENTS IN USE

Tests performed on third (1/3) remaining re agents brought from laboratories of KANDI, Malanville and N'dali hospitals shown that the average values of normal and pathological control serum are statistically and significantly different from the true expected value for the three reagents (glucose, calcium, transaminases) and for the two selected Chromatest and Biolabo variety.

Student statistics (Table IX) test values for the three parameters of the three areas are all greater than the critical values. There is a significant statistically difference between the true value and the mean value of the tests. We can say that the average dosage are statistically inaccurate compared to the target control values. Students test values of interseriel and intraseriel variation coefficients shown that the analytical process is lack of precision and therefore shown reagents stability problems.

3-3 Dynamic deterioration of calcium and transaminases reagents in use at Parakou, Kandi and Malanville hospitals

3-3 Dynamic deterioration of calcium and transaminases reagents in use at Parakou, Kandi and Malanville hospitals

These two figures (2/3) illustrated growing deterioration of reagents in use when going southen to northen (Parakou to Malanville) and concerns mainly transaminase and calcium. Deterioration were obtained with opened reagents.

3-4 Microbiological degradation of Chapman and Mac conckey

Environmental conditions also affect bacteriological culture causing distur bances on valid diagnostic indicators.

Table I : Various reagents used

Settings	Glucose		Calcium		Transaminases	
brands	**CROMATEST**	**BIOLABO**	**CROMATEST**	**BIOLABO**	**CROMATEST**	**- BIOLABO**
Lot	**REF 1129005 2 x 50 mL**	**Ref LP80209**	**Cromatest : REF 1115000**	**Biolabo REF 80004**	**Chromatest : GOT : REF1105000 GPT : REF1109000**	**Biolabo GOT: REF 80025 GPT**

Table II: Statistical values for the analytical and stability process validation of the normal serum control (SCN)

Reagents		Accuracy: SNA targetaverage value	Accuracy: Average test value	Precision: Student's t test	Precision: CV intra-serial	Precision: CV inter-serial
Glucose						
glucose Chromatest		92.10	92.9	2,106	1.23	1.17
glucose Biolabo		92.10	92.8	1,831	1.40	1.32
Calcium						
calcium Chromatest		87	86.6	1,219	1.23	1.17
calcium Biolabo		87	86.3	1,393	1.89	1.80
Transaminases						
Transaminase Chromatest	GOT n = 5	43.4	42.9	1,309	1.99	1.78
	GPT n = 5	39.3	40.9	2,550	3.51	3.14
Transaminase Biolabo	GOT n = 5	43.4	42.6	2,169	1.94	1.73
	GPT n = 5	39.3	38.5	1,600	2.90	2.60

Risk α = 5%, df = 9, | t | = 2,262 ; Risk α = 5%, df = 4, | t | = 2.77

Table III: Statistical values for the validation, of the analytic process and stability of the reagents in the opening with the pathological control serum (PCS)

Reagents		Accuracy: SCP targetaverage value	Precision: Average test value	Precision: Student'st test	Precision: CV intra-serial	Precision: CV inter-serial
Glucose chromatest	Gluc.C	276	274.9	1,966	0.61	0.58
Glucose Biolabo	Gluc B	276	276.5	1,342	0.43	0.40
Calcium Chromatest	Cal C	120	119.4	1,703	1.00	0.94
Calcium Biolabo	Cal B	120	120.9	1,112	2.14	2.03
Transaminase Chromatest	GOT C not 5	167	168.3	1,998	0.84	0.75
	GPT C not 5	119	119.0	0,000	1.31	1.18
T Transaminases Biolabo	GOT B No: 5	167	166.5	0.596	1.22	1.09
	GPT B No: 5	119	118.2	2,424	0.64	0.57

Risk α = 5%, df = 9, | t | = 2,262 ; Risk α = 5%, df = 4, | t | = 2.776

Table IV: Statistical values of the stability study during reagents in use in laboratory of Parakou hospital. Test on Normal Serum Control (SCN)

Reagents		Accuracy: SNA targetaverage value	Accuracy: Average test value	Precision: Student t test	Precision: CV intra-serial	Precision: CV inter-serial
glucose chromatest		92.10	94.6	9.913	0.85	0.81
glucose Biolabo		92.10	114.7	55.667	1.12	1.06
calcium Chromatest		87	72.8	31.122	1.97	1.87
calcium Biolabo		87	99.5	47.431	0.84	0.80
Transaminase Chromatest	GOT n = 5	43.4	49.7	18.909	1.50	1.34
	GPT n = 5	39.3	44.3	17.226	1.48	1.32
Transaminase Biolabo	GOT n = 5	43.4	25.9	60.381	2.50	2.24
	GPT n = 5	39.3	28.78	44.297	1.85	1.65

Parakou-N'Dali Sanitary area

Table V: Statistical values of the reagent stability study during reagents in use in laboratory of Parakou hospital. Test on pathologic serum control (SCP)

		Accuracy		Precision	
Reagents	SCP target average value	Average test value	Student t test	CV intra-serial	CV inter-serial
glucose chromatest	276	268	15.715	0.56	0.53
glucose Biolabo	276	351	127.922	0.53	0.51
calcium Chromatest	120	112.04	18.345	1.22	1.16
calcium Biolabo	120	145.41	66.127	0.84	0.79
Transaminase Chromatest	GOT n = 5	167	148.26	29.720	0.95	0.85
	GPT n = 5	119	137.6	46.646	0.65	0.58
Transaminase Biolabo	GOT n = 5	167	116.8	102.470	0.94	0.84
	GPT n = 5	119	82.2	63.111	1.59	1.42

Risk α = 5%, df = 9, | t | = 2,262 ; Risk α = 5%, df = 4, | t | = 2.77.

Kandi sanitary area

Table VI: Statistical values of the stability study during reagents in use at KANDI hospitals laboratory, Test on Normal Serum Control (SCN)

		Accuracy		Precision	
Reagents	SNA targetaverage value	Average test value	Student's t test	CV intra-serial	CV inter-serial
glucose chromatest	92.10	89.31	6.632	1.49	1.41
glucose Biolabo	92.10	112.4	54,690	1.04	0.99
calciumChromatest	87	72.55	40.953	1.54	1.46
calcium Biolabo	87	121.67	118.064	0.76	0.72
Transaminase chromatest	GOT n = 5	43.4	46.3	4,827	2.90	2.60
	GPT n = 5	39.3	41.14	3,157	3.17	2.83
Transaminase Biolabo	GOT n = 5	43.4	15.9	94.884	4.08	3.65
	GPT n = 5	39.3	18.38	53.588	4.75	4.25

Table VII: Statistical values of the reagent stability study during reagents in use at Kandi hospitals laboratory. Test on pathologic serum control (SCP)

		Accuracy		Precision	
Reagents	SCP target average value	Average test value	Student t test	CV intra-serial	CV inter-serial
glucose chromatest	276	264.2	40.607	0.35	0.33
glucose Biolabo	276	341	169.269	0.36	0.34
CalciumChromatest	120	102.24	47.878	1.15	1.09
calcium Biolabo	120	161,26	117.255	0.69	0.65
TransaminaseChromatest	GOT n = 5	167	112.86	6,333	16.94	15.15
	GPT n = 5	119	112.1	8.953	1.54	1.38
TransaminaseBiolabo	GOT n = 5	167	56.6	276,000	1.58	1.41
	GPT n = 5	119	53	208.710	1.33	1.19

Risk α = 5%, df = 9, | t | = 2,262 ; Risk α = 5%, df = 4, | t | = 2.776

Malanville Sanitary area

Table VIII: Statistical values of the stability study during reagents in use at Malanville hospitals laboratory. Test on Normal Serum Control (SCN)

Reagents	Accuracy		Precision			
	SNA targetaverage value	Average test value	Student's t test	CV intra-serial	CV inter-serial	
Glucosechromatest	92.10	117.19	99,100	0.68	0.65	
glucose Biolabo	92.10	122.4	81.631	0.96	0.91	
calciumChromatest	87	61.51	80.836	1.62	1.54	
calcium Biolabo	87	117.97	80.742	1.03	0.98	
Transaminase chromatest	GOT n =5	43.4	35.04	23.589	2.26	2.02
	GPT n =5	39.3	42.08	8,688	1.70	1.52
Transaminase Biolabo	GOT n =5	43.4	11,04	73.607	8.53	7.63
	GPT n =5	39.3	10.83	93.914	6.26	5.60

Table IX: Statistical values of the reagent stability study during reagents in use at Malanville hospitals laboratory. Test on pathologic serum control (SCP)

Reagents	Accuracy		Precision			
	SCP targetaverage value	Average test value	Student t test	CV intra-serial	CV inter-serial	
glucose chromatest	276	255.3	61.782	0.41	0.39	
glucose Biolabo	276	353.86	243.471	0.29	0.27	
calciumChromatest	120	90.4	135.324	0.79	0.75	
calcium Biolabo	120	159.01	88.545	0.88	0.83	
TransaminaseChromatest	GOT n = 5	167	145.58	35.291	0.93	0.83
	GPT n = 5	119	122.04	6.787	0.82	0.73
Transaminase Biolabo	GOT n = 5	167	65.24	200.495	1.74	1.56
	GPT n = 5	119	62.92	338.792	0.59	0.53

Risk α = 5%, df = 9, | t | = 2,262 ; Risk α = 5%, df = 4, | t | = 2.776

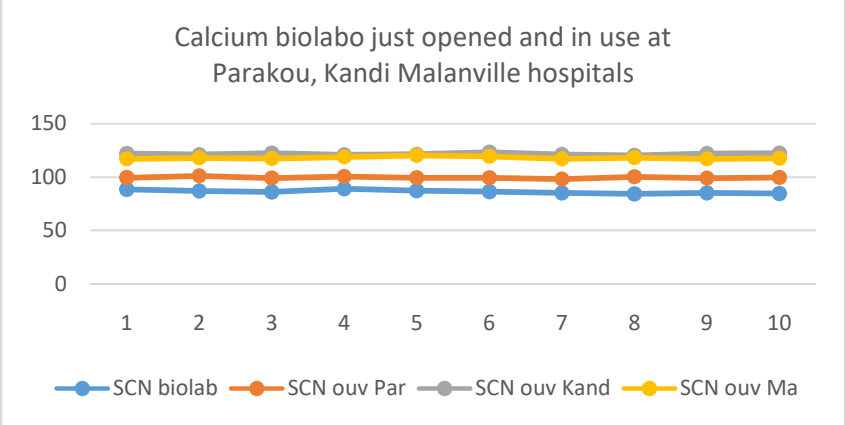

Figure 2: Calcium biolal deterioration in Parakou, Kandi and Malanville hospitals

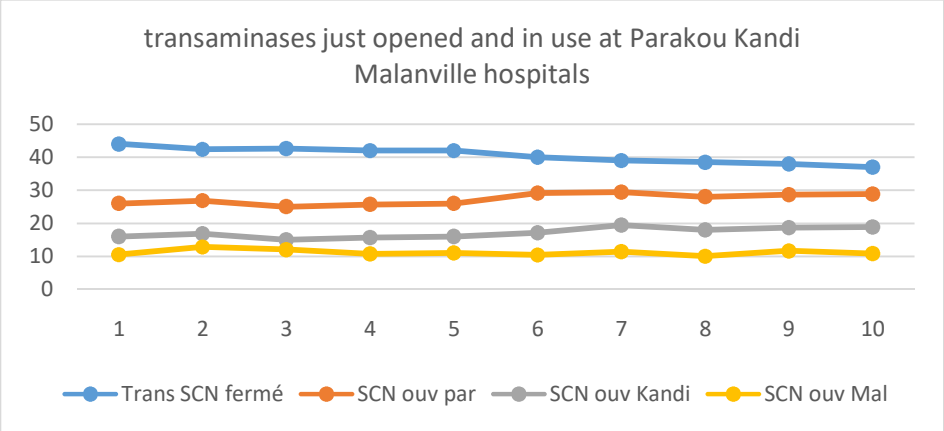

Figure 3: Transaminase deterioration inParakou, Kandi and Malanville hospitals

Table X: Comparison between moistened Chapman and appropriate Chapman

Characters	Appropriate chapman	Unappropriate chapman
Aspect	Fine light yellow powder	Wet compact mass of yellowish color
Appearance of agar	Gelatinred	Gelatin less consistency of yellowish color-disappearance of red phenol
Selectivity	Do not let grow the strain (ATCC # 25922) Escherichia coli but the strain (ATCC # 25923)Staphylococcus aureus	Do not let grow the strain (ATCC # 25922)Escherichia coli but the strain (ATCC # 25923)Staphylococcus aureus
Sterility (incubation one day before use)	No strain of growth	No strain of growth
Fertility	Growth Significantstrain (ATCC # 25923)*Staphylococcus aureus*	Significant growth of the strain (ATCC # 25923)Staphylococcus aureus
Biochemical characters	Catalase + Glutamine degradation (yellowingof the medium) DNase +	No glutamine metabolism (not noticeable yellowing.) The golden Staph.non appear golden

Table XI: Comparison of moistened Mac Conkey andappropriate Mac Conkey

Characters	Appropriate mac conkey	Unappropriate mac conkey
Aspect	Light yellowpowder	Wet compact red mass clear, tacky mass
Appearance of agar	Light redgelatin	Gelatine very inconsistent. Can be penetrated by the platinum handle
Selectivity	Do not let grow the strain (ATCC # 25923) Staphylococcus but the strain (ATCC # 25922)Escherichia coli	No consistency to withstand seeding flood Seeding No bacterialgrowth
Sterility (incubation day before use)	No strains growth	No strain of growth
Fertility	Significant growth of strain ATCC # 25922) Escherichia coli	Seeding by flooding : No bacterial growth.
Biochemical characters	Metabolizes lactose	No bacterialgrowth.

4 - DISCUSSION

Tests carried out on just opened reagents and reagents in use shown that environment working and climate have effect on reagents management and were confirmed by tests results performed just when reagents are openedcompared to test on reagents in use brought from hospital's laboratories. Our results are confirmed by Camara Cisse and *al.* work [3]. Just

opened, reagents parameters values are close to target equipment control values. Student T test comparison shown that there is no significant difference between the real values of the control equipment and our obtained average values for α risk of 5%. Intraserial and interserial variation coefficients were below 2%.

Tests on reagents used to tiers and brought from differents laboratories confirmed encountered stability problems of areas. Most of obvious problems are high temperatures of Kandi and Malanville areas, harmattan dust of the laboratories where analysis were done, intrinsically risks of reagents and using risks as identified by Bauthier-Loiseau [4]. Test made on calcium reagent during use whatever the areas revealed unstable character because of high temperatures risks and laboratories environment dust. All founded values are outside confidence area (mean ± 3s) with deviations ranging from 10 mg/L to 23mg/Lfor calcium, and from 15 to 40 IU/L for transaminases. These results are similar to Vassault and *al.* [5] which explains that calcium assay was based on chromophore complex formation and risk factor could change reagent color intensity and substrate value. This observation is valid for all other tested reagents (transaminases and glucose).Despite of relative stability and integrity of transaminases and glucose Chromatest reag -ents, all of them were disturbedby user's management, hot climateand environment dust.

False results used to regular sick patient may worsen his bad health. Hypocalcemia do to fetal subtraction in a pregnant woman may moveat last third years of the gestation and shown calcium homeostasis mechanisms involving 1.25 DOH allowing 24 hours to obtain intestinal absorption increased and PTH which increases bone resorption after 48 hours [6].

These signs associated with presumptive diagnosis must be confirmed by laboratory analysisfor people received in consultation. And as soon as these falsely elevated values obtained in a context where reagent quality were not guaranteed, it is clear that the clinician could not in any case restore calcium balance, same applies to transaminases. Instability found were confirmed by Sokou [7] results after maintained transaminase reagents eight hours at ambient temperatures of 28°C, showing that Spinreact brands transaminase are 6UI / L differences for the same specimen. And as stated in the erroneous calcium results case, transaminasetest poorly dosed have great impact on cell injury of the liver, heart, muscle and kidney of sick patientmanagement. Transaminases analysis should be falsely low

forcing clinician to take inappropriate decisions. These both observations confirm that, reagents integrity is related to their intrinsic chemical characticks. This intrinsic reagents stability character are explained with glucose Chromatest reagents which resist to disintegration when submitted to environment risks such as hot temperature, environment dust and laboratories management.

Hot climate and heat Harmattan starting from south Borgou (Parakou) to the north of Alibori (Malanville) showing dynamics deterioration of calcium and transaminase reagents were justified by figures 1 and 2. Climatic factor cannot be only responsible for this process, infrastructural problems and inadequate equipment such as cold chain, lack of air conditioning, are also responsibleof deterioration factors. Investigation on themedical laboratories mapping of Benin [8] shown that 4.2% of laboratories haven't internal tools quality control and constituded a major lack to good biological reagent management and to analytical process validation.

Reagents for germ growing are very sensitive to hygroscopy and to hot temperature. Variation in environmental factors changed visibly physical characters but chemical changes would be observed through sterile bacterial growing, or insignificant germ grow, making impossible bacterial identification. For example Staphylococcus mannitol apparently plus became mannitol least because of moisture which degraded glutamine removing red blow phenol color characteristic of the growing germ reagent [9].

Sterile growth observed for all germ put apparently in inadequate Conckey Mac shown that component which promoted nutritious growth are distorted. These reagents that are still used in our laboratories because of economic criteria shown clearly wrong diagnosis. That situation generated an additional cost to the sick patient, an extension duration of sick and antibiotic resistance problems. It is also difficult for african laboratories to assess risk factors because inspection procedure of our laboratories as stated in the medical biology laboratory document are very expensive [10].

CONCLUSION

Validity of health products expiry dates in general and diagnostic reagents in particular are closely linked to the need to conserve these products according to manufacturer's specifications

Environmental work dust, hot climate, harmattan climate, bad laboratory management, bad laboratory equipment have effect on reagents stability. It is therefore important to kept away environment contaminants which may deteriorate reagents in order to provide

valid biological analysis results for good patient health management.

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