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Evaluating access to Malaria Rapid Diagnostic Test kit and Artemisinin-based Combination Therapy and the Quality of Treatment Practice among Over-the-Counter Medicine Sellers at the District Level in the Brong Ahafo Region - Ghana

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Abstract

Background: Accurate diagnosis and appropriate treatment of malaria with the recommended antimalarials are crucial in the fight against malaria. This study evaluated the availability and sources of ACT and RDT kits among the Over-The-Counter (OTC) medicine sellers in the Pru, Sene and Atebubu-Amantin Districts of Ghana.

Method: A cross-sectional descriptive study was conducted using a structured questionnaire. Sixty-two OTC medicine sellers were randomly selected, informed consent sought and interviewed on access to RDT kit, ACT and Training in malaria diagnosis. Data entry, editing and analysis was done using SPSS Ver 22.

Results: The study revealed that 26.2% of respondents use the RDT kits to diagnosis and confirm suspected malaria. 94.1% respondents who had malaria RDT kits at their shop purchased them from the NMCP and Pharmacy shops. 95% of respondents had in stock at least one of the 3 recommended ACT - artesunate amodiaquine, artemether lumefantrine, and/or dihydroartemisinin piperaquine. 65.6% of respondents often recommend artemether lumefantrine to patients to treat uncomplicated malaria. The average wholesale and retail prices of the Affordable Medicine Facility malaria (AMFm) branded ACT were higher than the approved suggested retail prices.

Conclusion: Most OTC medicine sellers do not comply with the national antimalarial drug treatment policy. 85% of OTC medicine sellers purchase their AMFm branded ACT from second-line buyers at relatively higher price.

Recommendation: Regular training of OTC medicine sellers on malaria control and easier access to quality and affordable malaria RDT kits and ACT would help improve malaria control at the community level.

Keywords: Malaria, ACT, RDT, OTC medicine seller, Pru, Sene and Atebubu-Amantin.

Introduction

The World Health Organization (WHO) guidelines for treatment of malaria recommends parasitological testing of all suspected cases of malaria in all settings with either microscopy or rapid diagnostic test (RDT) kits (WHO malaria treatment Guidelines, 2015). Per the treatment guidelines, Artemisinin-based Combination Therapy (ACT) is the recommended medicine for the treatment of uncomplicated malaria. However access to these appropriate malaria diagnostic tools and treatment protocols remains a challenge in resource constrained settings. Treatment is further compromised by the widespread use of anti-malarial drugs for all fevers and by incorrect dosing (Danquah, 2010).

In Ghana, malaria contributes significantly to ill health in the general population and contributes to overall mortality especially in infant and maternal related deaths, accounting for about 40-50% of outpatient consultations in hospitals and clinics. It also contributes directly to low productivity, reduced school attendance and poor academic performance among school children in Ghana.
As part of efforts to improve access to prompt treatment, particularly in isolated rural areas, community-level interventions which seek to strengthen home management of children with malaria are gaining importance. Private pharmaceutical facilities are the main points of call for the management of uncomplicated malaria in sub-Saharan Africa (Danquah, 2010). In Ghana the community-based drug retail outlets popularly called over the counter (OTC) medicine shops are well-known first points of call for malaria treatment.

**Over-the-counter (OTC) medicine sellers and malaria control in Ghana**

In Ghana, OTC Medicine Sellers’ shops are the principal source of medicinal products for the Ghanaian rural population. A Licensed OTC Medicine seller is a private provider who has obtained the appropriate license from the Pharmacy Council, which authorizes him or her to engage in the retail of only Over-the-Counter drugs (Class C or OTC drugs) at a premise or location specified in the license (Pharmacy Act, 1994 -ACT 489). According to the Ghana National Over-the-Counter Medicine Sellers Association there are an estimated 13,000 registered OTC Medicine Sellers in Ghana. Selection exercises conducted by FHI 360 malaria team in 2015 indicated there are over 100 OTC medicine sellers in the Pru, Sene and Atebubu-Amantin districts. Studies conducted by Goodman et al (2007a), Smith (2004) and Van den Boom et al (2004), on health seeking behaviour among Ghanaians suggested that, community pharmacies and licensed chemical shops were usually the first port of call for health advice and treatment for many individuals with symptoms suggestive of malaria. The Mobilize against Malaria (MAM) project baseline report in 2008 indicated that OTC Medicine Sellers are recognized as the first-line or only source of consultation for 60 percent of people seeking health care in general. The explanation given for this observation was that, those community-based retail outlets operated for long hours, were not charging consultation fees, and patrons did not have to wait for long hours before having access to practitioners to purchase antimalarials. The retail practitioners were also perceived to be friendlier, and payments for anti-malaria services were flexible for known community members (Ahorlu et al 1997, Goodman et al 2007a, Smith 2009b). Knowledge and practice of OTC medicine sellers in malaria management, prevention and control are thus crucial to the fight against malaria at the community level. To date, little research has been done to document the knowledge and practice on malaria case management among OTC medicine sellers and its effect on malaria control in Ghana. This means the role of Over-the-Counter Medicine Sellers in Ghana has less been exploited. The objective of this study was to assess the malaria knowledge and practice among over-the –Counter Medicine Sellers in the Brong Ahafo region of Ghana.

**Rapid diagnostic test (RDT) kit for malaria diagnosis**

Rapid Diagnostic Test (RDT) kits are quick diagnostic tests that use antibodies to detect malaria parasite antigens in a blood sample. Prompt, accurate diagnosis of malaria is part of effective disease management (WHO malaria treatment Guidelines, 2015). The current malaria treatment guidelines strongly recommends parasitological testing of all malaria cases under all settings with either RDT (especially under resource -constrained settings) or microscopy (if available). WHO strongly advocates the policy of “Test, Treat and Track” to improve quality of care surveillance (WHO, 2015). This has been driven by several factors, including the fact that a large proportion of patients with fever are being given antimalarial medicines when they have other, potentially serious ailments which are missed (Ansah et al, 2015) and the wastage of relatively expensive antimalarial medicines on patients without malaria.

The current malaria treatment policy recommends that OTC medicine sellers use RDT to confirm suspected malaria cases before treatment of uncomplicated malaria or referring the complicated ones to the appropriate health facility. However not all OTC medicine sellers have been trained in the use of malaria RDT (unpublished information from Sene West Secretary, OTC Medicine Sellers Association). Interviews with some trained OTC Medicine Sellers indicated that most of the trained ones do not have the RDT kits. They attributed low patronage by clients, relatively high cost and lack of constant supply as the reasons for the unavailability of the kits.
Access to antimalarial medicine

Medicines are useful in promoting health, preventing and managing diseases but can be harmful when used inappropriately (Danquah, 2010). A baseline survey conducted by Health Partners Ghana in the Mobilise Against Malaria (MAM) project in five districts in Ashanti Region found that the commonest anti-malarial medicines sold in the LCS (now OTC Medicine Sellers) shops were all mono-therapies, with Sulphadoxine-Pyrimethamine being the most commonly stocked medicine (82% of LCS), followed by Amodiaquine syrup (73.9%), and then Artesunate tablets (50.9%). Combination therapy of any form was sold less frequently in the LCS shops; Artemether-Lumefantrine (37.9%); Artesunate – Amodiaquine (19.9%) – (MAM Project Baseline report, 2008). In rural areas in particular, most of the poorest often access antimalarials through the private drug retail sector, largely because of the transport cost and opportunity cost (things people could otherwise spend money on) of accessing healthcare (Ansah et al, 2015). In recognition of this, several schemes, including the Affordable Medicines Facility for malaria (AMFm) have been deployed to ensure that effective antimalarial medicines are available through private outlets that poorer patients use (Arrow et al, 2004).

Methods

Study design

A cross-sectional descriptive study was conducted in the Pru, Sene and Atebubu-Amantin districts of the Brong Ahafo Region of Ghana among Over-the-Counter Medicine Sellers to evaluate the availability and the source of Artemisinin-based Combination Therapy (ACT) and malaria rapid diagnostic test (RDT) kit.

Study area

Pru, Atebubu-Amantin and Sene districts are rural districts in the Brong Ahafo region of Ghana. They are predominately rural with large tracts of forest, many water bodies, high vector density for malaria, poor sanitation, with communities that are generally at high risk of malaria. The districts are hyperendemic for malaria with prevalence of 70-90% (NMCP report 2008). There are few Health facilities in the districts, this also accounts for high patronage of the services of the OTC medicine sellers. There are over 100 OTC sellers operating in these rural districts.

Sampling method

A list of Over-the-Counter Medicine sellers was obtained from the Association Executives and also FHI 360 OTC Medicine sellers identification exercise indicates a total of 106 OTC Medicine sellers (Pru -21, Sene -34 Atebubu-Amantin-51). Sixty-two (62) OTC Medicine Sellers was randomly selected, informed consent was sought and interviewed.

Data collection tools, processing and analysis

Structured questionnaire and informed consent form

Informed consent was sought from the OTC Medicine sellers after which structured questionnaires were administered to the respondents at the OTC Medicine Shop. The interviews were conducted by four trained data collectors from March 5 to 12, 2016. The interviews sought to elicit information on the knowledge and skills of the OTC medicine sellers in malaria transmission and prevention, tools or criteria for diagnosis, treatment regimen, availability of Rapid Diagnostic Test kit, sources and availability of Artemisinin-based Combination Therapy in the shop and malaria training participated within the past two years.

Data processing and analysis

The raw data was initially captured in Microsoft excel and Statistical Package for Social Sciences (SPSS) version 22 was used to clean/edit and analyze the data quantitatively. The necessary plots and tables were prepared and inferences drawn from the analysis.
Ethical considerations

Ethical approval (CHRPE/AP/038/16) was sought from the Committee on Human Research, Publication and Ethics (CHRPE) - the Kwame Nkrumah University of Science and Technology (KNUST). Permission was also sought from the Pru, Sene West and Atebubu-Amantin District Directors of Health Services, and the Districts OTC medicine sellers Associations. The research participants were OTC medicine sellers who gave their informed consent and voluntarily participated. Privacy, confidentiality and anonymity were assured and ensured. Participants were not compensated for the information provided.

Results

Demographic characteristics of respondents

Sixty two (62) respondents consented and were interviewed. About 47% of the respondents were shop owners and 53% were shop assistants. Majority of the respondents were males, 52 (83.9%). The females constituted 16.1% (10) of the total respondents, and accounted for 27.3 % (9) of the shop assistants and 3.4 % (1) of the shop owners. Most of the respondents were within the age group 20 – 39 years (58.1%). There was no OTC medicine seller under 18 years of age. 23 of the respondents, representing 37.1% were within the age range 40 – 59 years, with only one person (1.6%) been above 60 years (62 years). 39 respondents (63%) had participated in at least one malaria training workshop organized by the Pharmacy council and/or its partners within the past two years ;2013, 2014 and 2015,. (Table 1)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age group (years)</th>
<th>Training in Malaria control participated in within the past 2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
<td>18 - 19</td>
</tr>
<tr>
<td>Frequency (N=62)</td>
<td>52</td>
<td>10</td>
</tr>
<tr>
<td>Percentage</td>
<td>83.9%</td>
<td>16.1%</td>
</tr>
</tbody>
</table>

Malaria diagnosis practice

Malaria diagnosis

54.1% (33/61) and 26.2% (16/61) of respondents use clinical symptoms and Rapid Diagnostic Test (RDT) kits respectively to diagnose malaria. About 5% (2) use “Malaria Job Aid” to diagnose malaria and almost 8% (3) use “Assumption” to diagnose malaria. Over 30% (7 out of 23) of the respondents who had not participated in any malaria training within 2013 and 2015, use Assumption to diagnose malaria. None of the respondents who had never participated in malaria training within 2013 and 2015 used Malaria Job aid to help diagnose malaria. There was a significant difference ( p = 0.009 ) between the responses of respondents who had participated in malaria training within 2013 and 2015 and those who had not in relation to the following methods of diagnosing malaria; “RDT” “Assumption” and “Malaria Job aid” See Table 2 for details.

Rapid diagnostic test kit (RDT) training, possession, source and price

Sixty-one (67.2%; n=61) had been trained on how to use malaria RDT kit to diagnose malaria. Out of the 61, only 27.9% (17) had malaria RDT kit at the Shop at the time of the interview. Some reasons
given for the unavailability of the malaria RDT kits at the time of the interview included: “low stocks”, “low patronage of the RDT by client due to price concerns”, and “the inconsistency in the supply of RDT by the suppliers”. 94.1% (16/17) respondents who had RDT bought their malaria RDT kits from Pharmacy shops and the National Malaria Control Program (NMCP). Only one person procured the kit through the District OTC Medicine Sellers Association. The average whole sale price per cassette of a malaria RDT kit was One Ghana cedi forty five pesewas; GH₵ 1.45±0.16 (equivalent to USD $ 0.37±0.04) and the average retail price per cassette of a malaria RDT kit was Two Ghana cedi four peswes GH₵ 2.04±0.13(USD $ 0.53±0.03) - See table 2 [Exchange rate GH₵ 3.85 = USD $ 1.00]
Table 2. OTC medicine sellers’ response on malaria diagnosis and access to RDT kit

<table>
<thead>
<tr>
<th>Malaria diagnosis practice</th>
<th>Trained in how to use malaria RDT</th>
<th>Had Stock RDT at shop at time of interview</th>
<th>Source of RDT kit *N1 = 17</th>
<th>Price per RDT cassette (GHC ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Use Clinical symptoms</td>
<td>Use RDT kit</td>
<td>Presumptive/assumption</td>
<td>Use Malaria job aid</td>
</tr>
<tr>
<td>Number of respondents (N=61)</td>
<td>33</td>
<td>16</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Percentage</td>
<td>54.1%</td>
<td>26.2%</td>
<td>16.4%</td>
<td>3.3%</td>
</tr>
</tbody>
</table>
Malaria treatment practice

92% (57/62) of the respondents mentioned ACTs as the recommended drugs for treating uncomplicated malaria. Apart from ACTs, respondents also stock and dispense other antimalarial medicines. 43.5% (27/62), 38.7% (24/62), 30.65% (19/62) and 26% (16/62) indicated/reported they stock and dispense quinine (syrup for infants, tablet for adult), monotherapies (specifically amodiaquine syrup for infants), Sulphadoxine-Pyrimethamine (SP) and herbal antimalarial mixtures for treating uncomplicated malaria respectively. No respondent indicated Chloroquine for treating malaria (Table 3). About 65.6% (40/61) of respondents often recommend AL as the first choice for treatment of uncomplicated malaria. Reasons attributed to this included, greater tolerance by patients in comparison to other ACTs, clients’ preference, effectiveness, availability and accessibility on the market. 24.6% (15/61) of respondents often recommend AS/AQ to patients due to its effectiveness in treating uncomplicated malaria. 6.6% (4/61) often recommend SP for treating uncomplicated malaria. Attributable reasons included; non-availability of subsidized ACTs and the high cost of available ACTs and insistence by some clients on SP usage for malaria treatment (Table 4).

69.4% (43/62) of respondents dispense antimalarial based on the patient’s age, 19.4% (12/62), 4.8% (3/62) and 4.8% (3/62) of respondents dispense antimalarial based on patient’s weight and age, actual weight of patient and on Clinicians’ prescriptions respectively (Table 4).

Fifty-five (90 %) of 61 respondents who often recommend ACTs for patients, described the treatment regimen of both AL and AS/AQ as; “4 tablets twice daily (morning and evening) for 3 days for adults” and “2 tabs twice daily (morning and evening) for 3 days for children”. Barely 4 out of 61 respondents stated that “the first dose of AL for day one should be taken 8hours interval”.

Four (4) respondents who often recommend SP to patients, described the treatment regimen of SP as “3 tablets single dose”.

Table 3. Antimalarial medicine stock and sell by respondent

<table>
<thead>
<tr>
<th>Decision on dosage of medicine for treating malaria</th>
<th>Number of Respondent (N=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base on patient’s age</td>
<td>43 (69.4%)</td>
</tr>
<tr>
<td>Base on estimated weight and age of patient</td>
<td>12 (19.4%)</td>
</tr>
<tr>
<td>Base on actual weight</td>
<td>3 (4.8%)</td>
</tr>
<tr>
<td>Base on Clinician’s prescription</td>
<td>3 (4.8%)</td>
</tr>
<tr>
<td>None of the above</td>
<td>1 (1.6%)</td>
</tr>
</tbody>
</table>

Artemisinin-based combination therapy (ACT) stock, sources and prices

Type of ACT stocked at shop

At the time of the interview 58 (95%) respondents had at least one of the three ACTs (AS/AQ and/or AL and/ or DP) in stock. Over 44% (27) of respondents had only AL. 42.6% (26) had both
AS/AQ and AL, 3.2% (2) had all the three recommended ACTs (AS/AQ, AL and DP). Three respondents had only AS/AQ and 3 respondents (1 trained OTC Medicine seller and 2 non trained OTC medicine sellers) had none of the three ACTs. No shop had only DP in stock (Figure 1)

![ACT available at Respondent’s shop](image)

Figure 1. ACT available at Respondent’s shop

The figure shows the response of OTC Medicine sellers on relative stock of Antimalarials in the shop at the time of interview. 95% respondents had at least one of the three ACTs (AS/AQ and/or AL and/or DP) in stock. 44% of respondents had only AL. 42.6% had both AS/AQ and AL, 3.2% had all the three recommended ACTs (AS/AQ, AL and DP).

AS - Amodiaquine syrup; AL - Artemether-Lumefantrine; DP - Dihydroartemisinin Piperaquine

**Sources and suppliers of ACT**

Fifty-two respondents, representing 85.2% (52/61) purchase their ACTs from Pharmacies and Pharmaceutical Companies in Kumasi, the capital city of the Ashanti region - Ghana. 10 out of the 52 (19.2%), 7 out of the 52 (13.5%), 5 out of 52 (9.6%), another 5 out of the 52 (9.6%) and 2 out of the 52 (3.8%) respondents buy their ACTs from PokuPharma Limited, Oson’s Chemist Limited, Tobinco Pharmaceuticals Limited, Dannipharma Limited and Kinapharma Limited respectively. Twenty-three of the 52 (44.2%) respondents did not mention the name of the Pharmacy or Pharmaceutical Company they buy their ACTs from in Kumasi. Reasons given was that “they do not have one particular Pharmacy in Kumasi they buy their ACTs from. Also sometimes the shop owners buy them from Kumasi”. Five (8.2%) and two (3.3%) respondents purchase their ACTs from the Kwapong Pharmacy at Atebubu, Atebubu-Amantin district and Rhema Pharmacy at Yeji, the Pru districts respectively (Figure 2). Per the Affordable Medicine Facility-malaria (AMFm) list of first-line buyers in Ghana (AMFm First-line buyer list, 2013) Oson’s Chemist Limited and Tobinco Pharmaceuticals Limited are among the accredited first line buyers of the affordable ACTs in Ghana. The remaining Pharmacies and pharmaceutical companies are not; therefore their wholesale prices and retail prices of ACTs may be quiet higher than that of Oson’s Chemist and Tobinco Pharmaceuticals Limited. According to Global Fund August 2010 Report, the Suggested Retail Price (SRP) of the AMFm branded ACTs was no more than GHC1.50 (equivalent to USD $1.00 then).
85.2% of respondents purchase their ACTs from Pharmacies and Pharmaceutical Companies in Kumasi, 19.2%, 13.5%, 9.6%, and another 9.6% and 3.8% respondents buy their ACTs from PokuPharma Limited, Oson’s Chemist Limited, Tobinco Pharmaceuticals Limited, Dannipharma Limited and Kinapharma Limited respectively. 44.2% respondents did not mention the name of the Pharmacy or Pharmaceutical Company they buy their ACTs from.

**Average wholesale and retail prices of ACTs**

The wholesale price range and retail price range of AS/AQ were GH₵1.50 - 7.00 (USD $0.39 -1.82) and GH₵3.00 -12.00 (USD $0.78-3.12) respectively. The average wholesale and retail prices of AS/AQ by respondents were GH₵2.85±1.28 (USD $0.74±0.33) and GH₵5.18±2.18 (USD $1.35±0.57) respectively. The wholesale price range and retail price range of AL were GH₵1.00 - 7.00 (USD $0.26 -1.82) and GH₵3.00 -12.00 (USD $0.78-3.12) respectively. The average wholesale and retail prices of AL by respondents were GH₵2.97±1.23 (USD $0.77±0.32) and GH₵5.51±2.21 (USD $1.43±0.57) respectively. The wholesale price range and retail price range of DP were GH₵5.00 - 8.00 (USD $1.3 -2.08) and GH₵9.00 -14.00 (USD $2.34 -3.64) respectively. The average wholesale and retail prices of DP by respondents were GH₵6.50±2.12 (USD $1.69±0.55) and GH₵11.5±3.54 (USD $2.99±0.92) respectively. Whiles there were no significant differences between wholesale prices of AS/AQ and AL and retail prices of same, DP showed a nearly 56% difference between the wholesale price and retail price (Figure 3)

![Figure 2. Sources/Suppliers of ACTs to Respondents](image-url)
Key: W.P - Wholesale Price R.P - Retail Price Exchange rate GH₵ 3.85 = USD $ 1.00 The figure exposes the cost implications of particular antimalarials. While there is little difference between AS/AQ and AL pricing at the wholesale and retail level, DP show nearly 56% difference between the wholesale price and retail price of DP.

Most patronized ACT by patients

Fifty (50) respondents, representing 86.2% (50/58) of the respondents indicated that AL is the most patronized ACT. Reasons for the high patronage of AL include comments like “it is very effective”, “it is always available” “it works fast” “it is affordable” and “patient tolerate the strength of AL”. 13.8% (8/58) also said AS/AQ is preferred because it very efficacious” and “cures malaria faster” (figure 4). 32.8% (20/61) respondents buy and stock ACT as and when it is stocked out, hence has no particular trend of stocking ACT at shop. 29.5% (18/61), 21.3% (13/61), 8.2% (5/61) and 3.3% (2/61) of respondents stock ACT weekly, monthly, bi-weekly and everyday respectively.

The figure indicates the high patronage of AL at 86% of respondents.
Discussion

Malaria diagnosis and treatment practice

This study revealed that about 74% of the OTC medicine sellers use either clinical symptoms or presumption/assumption of “trial and error” to diagnose malaria, with only 26.2% of them using the rapid diagnostic test (RDT) kit to diagnosis and confirm suspected cases of malaria. This is not in compliance with the WHO Guidelines for Treatment of Malaria, (2015). This guideline recommends that in all settings all cases of suspected malaria should be confirmed with a parasitological test i.e. microscopy or RDT. Danquah, 2010 made similar conclusions from a study conducted in the Ashanti Region. There was a significant difference (p<0.05) between the malaria diagnosis of OTC medicine sellers who had participated in malaria training within the past 2 years and those who had not. An OTC medicine seller who has participated in malaria training within past 2 years was more likely to use RDT to diagnose malaria than an OTC medicine sellers who has not.

This study revealed that majority (69.4%) of OTC medicine sellers decide on the dosage of antimalarial medicine for treatment based on the patient’s age. Only 19.4% and 4.8% of them decide on dosage based on the estimated weight and age rather than the actual weight of the patient. This is attributed to the fact that most OTC medicine sellers do have weighing scale. Hence very few treat malaria according to the recommended guidelines.

Antimalarial medicine stock and dispense to patients by OTC medicine sellers

Per the national antimalarial drug policy and the WHO Guidelines for Treatment of malaria, 2015, ACT is the recommended drug for the treatment of uncomplicated malaria. Monotherapies (artesunate, artemether, amodiaquine and lumefantrine), Sulphadoxine-Pyrimethamine (SP), quinine and herbal preparations are not recommended for the treatment of uncomplicated. This study showed that 95% of respondents stock and sell ACTs. Among the ACTs, Artemether Lume fantrine (AL) is the most (65.6%) respondents recommend to patient for malaria treatment, followed by Artesunate Amodiaquine (AS/AQ). However, as high as 43.5%, 38.7%, 30.7% and 26% of respondents stock and sell quinine, monotherapies, SP and herbal preparations respectively to treat uncomplicated malaria. Reasons stated by most of these OTC medicine sellers included the fact that whiles “some clients/patients insist on using the SP, monotherapies, herbal and the quinine”, “others could not afford ACT” and “they often run out of stock of the affordable medicines - facility-malaria (AMFm) branded ACT and are left with no option than to sell these ones”. This implies that the availability of ACT in an OTC medicine shop does not guarantee its use. This finding concurs with that of Danquah 2010, where 71.9% of Licensed Chemical Sellers’ (LCS) shops had only monotherapies among which 28.1% had only SP. There is therefore the need for effective and strong pharmacovigilance system and monitoring by the Pharmacy council and District Health team to ensure compliance. There is also the need to strengthen the existing systems and build effective structures that would ensure easier access to quality and affordable ACTs at the community level.

This study revealed that 90% of OTC medicine sellers could not explain appropriately the treatment regimen of the ACTs, especially the treatment regimen of AL. Most of them describe the treatment regimen of AL in the same way AS/AQ is taken. Barely 4% of OTC medicine sellers stated “the first dose of AL for day one should be taken 8 hours interval”.

ACT Stock, sources and prices

The study revealed that 95% of OTC medicine sellers had in stock at least one of the 3 recommended ACTs - AS/AQ, and/or AL and/or DP (See figure 1). A similar study by Danquah 2010 indicated that 94.1% of Pharmacies and 28.1% of LCSs had the recommended anti–malarial (ACT) available at their shops. 32.8% of respondents had no particular time schedule of re-stocking their shop with ACT. They stock ACTs as and when stocks are low. 65% of the respondents re-stocks their shops with ACTs at least every month. 44% and 43% of OTC medicine sellers indicated that AL and AS/AQ respectively are the most patronized ACT by patients.

According to the Global Fund August 2010 Report, the Suggested Retail Price (SRP) of the AMFm branded ACTs was no more than GHC1.50 (equivalent to USD $1.00 then). The study revealed that
the average retail prices: GH₵5.18±2.18 (USD $1.33±0.56) and GH₵5.51±2.21 (USD $1.42±0.57) (Figure 3) of the AMFm branded AS/AQ and AL respectively are far higher than the suggested retail price (SRP) of GH₵1.50 (USD $1.00) in August 2010.

85.2% of OTC medicine sellers purchase their ACT from Pharmacies or Pharmaceutical companies in Kumasi about 4 to 5 hours of 240 -250 km journey by public transport from Pru, Sene and Atebubu-Amantin districts. This study also revealed that only two of the Pharmaceutical Companies who supply ACTs to the OTC medicine sellers were AMFm First-line buyers of ACTs (AMFm first-line buyers list Ghana, 2013) hence the disparities in the wholesale and retail prices of the ACTs in the four districts (Figure 2 and 3).

Conclusion and recommendation

Majority of the OTC medicine sellers in the Pru, Sene and Atebubu-Amantin districts do not comply with the national antimalarial drug treatment policy. Only 26.2% of the OTC medicine sellers use RDT kit to test before treating malaria with over 94% (out of the overall 26.2%) buying their RDT kits from the National Malaria Control Program and Pharmacies.

Majority (85%) of OTC medicine sellers purchase their AMFm branded ACTs from second-line buyers at relatively high wholesale prices hence the high retail prices. Quite a high number (26% -43.5%) of OTC medicine sellers still sell non-recommended antimalarial. This means that the physical availability of the ACT in an OTC medicine shop does not guarantee access to effective therapy.

Regular training of OTC medicine sellers on malaria control and easier access to quality and affordable malaria RDT kits and ACTs would help improve malaria control at the community level.

Acknowledgment

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Principles, Assumptions, and Processes Established to Designate Biologics Assets as a Fast to FIH (First-in-Human) Program and the Associated Core Concepts for the Utilization of low and High-Risk Activities, Timelines and Functional Level Expectations

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**Abstract**

Biologics development represents a substantial advancement in the pharmaceutical industry because of their promise and huge success in the oncology, immunoscience, and cardiovascular disease areas. Prior to entering the marketed product development phase, each biopharmaceutical needs to go through series of stages that will allow or disallow the biologics asset to become a commercialized product. Each of those phases includes development planning and designing of studies to test relevant hypotheses to support the drug label if approved.

The current thesis will focus on the principles, assumptions, and processes that are established to designate an asset (biologics) as a targeted first-in-human program. First-in-human studies are included under phase 1 trials, where initial human exposure is initiated to the investigational new drug (IND). Phase 1 is critical since it affirms if a compound’s mechanisms of action in humans and its development can result in a potentially new drug entity.

Subsequently, step by step initiatives and processes from the perspective of different functional groups within the pharma will be revised to outline the staged procedures, methods, critical, and non-critical paths taken when a molecule is nominated as a clinical candidate. Overall alignment of deliverables will be presented between the different functional areas that partake in the first-in-human development.

Strategic changes to the biologics development process, cell line development with multiple candidate sequences, initial platform fit assessment for a process, analytical and formulation will be acknowledged. Platform strategy for drug substance production, as well as, drug product composition will be outlined along with boilerplates for analytical method development to fit or not fit the platform approach. The functional groups that will be reviewed will be; Discovery, Cell line development, Drug Substance process development, Formulation development, Toxicology, Quality, Drug Substance manufacturing, Drug Product manufacturing, Stability and regulatory.

**Keywords:** Fast to First in Human, Biologics, Development, Clinical.

**Introduction**

The main aim of the proposed study is to find out the principles, assumptions, and processes utilized in designating biologics assets as a Fast to FIH (First-in-Human) program, as well as, streamline the processes of high and low-risk activities using Fast to First-In-Human (FIH) model and their successful implementation and subsequent readiness for regulatory submission of the clinical trial application (CTA).

**Definition of the research problem**

Biologics can be used as oncology and immuno-oncology therapies (Bartlett, 2011). However, the development of biologics utilizes a lengthy and complex process (Pharma, 2017; Forum on Neuroscience and Nervous System Disorders, Board on Health Sciences Policy, & Institute of Medicine, 2014; Conner et al., 2014). The methods and processes used in the development of biologics have been changing as time goes. Earlier approaches are replaced by newer ones as technology and knowledge advance (Hojjat-Farsangi, 2014). However, it has been presumed that the principles, assumptions, and processes that are used to designate biologics assets as a Fast to FIH
(First-in-Human) program are still the same, despite the evident changes that have been witnessed in the pharmaceuticals industry (Atanasov, et al., 2015). In addition, organizations may have explored different approaches in the use of low and high-risk methods during the development of biologics. Consequently, there may have been changes in timelines, and functional level expectations. However, the associated changes have not been explored in terms of how they are incorporated in the development of biologics as a Fast to FIH (First-in-Human) program. The newer principles, assumptions and processes adopted in the manufacture and designation of biologics assets a fast to FIH program have not been evaluated. Therefore, given the existent scientific knowledge and an informational gap that needs to be filled. Additionally, it is necessary to explore the associated core concepts for the utilization of low and high-risk activities, timelines, and functional level expectations in the designation biologics assets as a Fast to FIH, given the changes.

Problems to be solved

The problems to be solved will test the following two hypotheses;

H10 There have been newer principles, assumptions, and processes established to designate biologics assets as a Fast to FIH (First-in-Human) program

H1A There have been no newer principles, assumptions, and processes established to designate biologics assets as a Fast to FIH (First-in-Human) program

H20 the associated core concepts for the utilization of low and high-risk activities, timelines, and functional level expectations in the designation of biologics assets as a Fast to FIH have changed significantly

H2A The associated core concepts for the utilization of low and high-risk activities, timelines, and functional level expectations in the designation of biologics assets as a Fast to FIH have not changed significantly

Biologics

Biologics have revolutionized the treatment of chronic diseases as they have enhanced the treatment of rheumatoid arthritis, psoriatic arthritis multiple sclerosis, and a variety of cancers (Mócsai, Kovács, & Gergely, 2014). Existing biologics include drugs such as Humira and Avonex. Biologics are defined as a virus, therapeutic, serum, toxin, antitoxin, vaccine, blood product or derivative or an arsphenamine applicable for prevention, and treatment or cure of disease or condition (Zhao, Ren, & Wang, 2012). Zimney (2008) observes that biologics are derived from living organisms. They are obtained from humans or animals. Those that rely on Biotechnology are referred to as second-generation biologics (Zimney, 2008). Biologics include monoclonal antibodies, cytokines and growth hormones. They have unique characteristics compared to small molecules (Zhao, Ren, & Wang, 2012).

The initial preclinical testing of biologics are used in vivo animal models, in vitro studies, and also computerized algorithms. First in human tests, (also called first-in-man) are tests that initially test drugs in humans, (Eisenhauer, Twelves, & Buyse, 2015). According to the FDA and ICH, there are specific guidelines for the introduction of biologics as first-in-man tests. For example, small molecule biologics that have selective toxicity as a mechanism are not required to have genotoxicity testing prior to first in human testing (Gad, 2011). Gad further notes that In Vitro studies using liver slices, microsomes, and in some cases hepatocytes, both from human and animals are used to show the drug metabolic profile before the initiation of clinical trials. For instance, in the testing of GC33, Zhu et al. (2013) conducted clinical trials to evaluate the safety, PK characteristics and preliminary efficacy of GC33 in patients with advanced HCC.

Development and designation of biologics assets as a Fast to FIH is a critical process that is associated with several risks and requirements that should be met. For instance, there has to be evidence of the effectiveness of the biologics assets to be developed in order to secure the approval of a license application. In addition, an accurate completion of the benefit/risk assessment of the molecular entity to be introduced is required (Kudrin, 2012). Safety of the product to be introduced is a requirement that should be fulfilled too. Therefore, before the development and designation of biologics assets as a fast to FIH, there has to be a sufficient number of well-controlled studies that
would act as a pilot test (Kudrin, 2012). The main assumption here is that the term "studies" is plural. Therefore, it is a requirement that at least more than one study is carried out, which will have to be controlled and randomized clinical trials. The use of such studies is to establish the efficacy of the biologics assets to be designated as a fast to FIH.

The first step of the process of designating a biologic asset as a fast to FIH involves execution of preclinical tests (FDA, 2017a). The first step is regarded as the initial process that is succeeded by other phases that become complex at every successive step. The reason for this is that each phase should enhance testing with successful clinical results to support approval for marketing and adoption of the biologic. In this case, the biologic molecule should successfully pass the preclinical tests and prove to be effective in oncology and immuno-oncology therapeutics. Therefore, according to the Institute of Medicine (US) Committee (2010), it is imperative that the proposed drug meets the safety and efficacy standards set by FDA. Each step of the process that is followed in the development of a biologic asset is expensive and risky. Consequently, well-endowed companies with sufficient resources such as biotechnology and pharmaceutical companies have the upper hand in developing and presenting biologics to be used for treatment (Wong, 2009; Waltz, 2014). It has also been established that a majority of the new drugs that have been developed as biologic assets have successfully passed the clinical testing steps and have been approved for marketing. However, it is presumed that the discovered biologics have the potential of becoming effective oncology and immuno-oncology therapies. The reason for this is that sometimes the chances of success are very low for some drugs development efforts while the costs of development are very high. Therefore, biotechnology and pharmaceutical companies focus on developing biologic assets that have the highest potential (Institute of Medicine (US) Committee, 2010). It has been noted that potential biologics meant to be therapies for life-threatening and rare diseases have been dropped at the very early stages of their development due to their failure in preclinical and clinical tests.

The next step of the development process of biologics for oncology therapy involves the execution of preclinical studies both in vitro and in animals to evaluate their safety potential and toxicity levels. It is through these preclinical studies that the potential effectiveness of the discovered biologic assets is reviewed (Institute of Medicine (US) Committee, 2010). The reason for this is that biotechnology and pharmaceutical companies would not like to invest in the development of therapeutic drugs that may be ineffective in the end because the process is very expensive and risky.

In the subsequent step of the development process, organizations that sponsor the development of biologic assets carry out additional clinical studies (FDA, 2017b). Such studies are meant to review and evaluate the evidence to guarantee that the drugs will evidently have no mutagenic problems (Institute of Medicine (US) Committee, 2010). Therefore, a biologic for oncology and immuno-oncology should not cause mutagenic alterations because this is very risky. In addition, the designed biologic should not have the potential of causing fetal malformations. Therefore, additional studies, beyond preclinical and clinical test ought to be carried out to ensure the potential, safety, and effectiveness of the proposed biologic drug.

In another step of the process, a determination of whether the biologic can be excreted by patients successfully (FDA, 2017c). The reason for this is that it is that a biologic drug should be excreted successfully as easily as it is absorbed or ingested by a patient (Institute of Medicine (US) Committee, 2010). Therefore, further studies and tests are required to ensure that the proposed biologic drugs meet this threshold. It is after the drug passes this test and meets the set standards that it can be passed on into the next stage of the development process.

According to Prueksaritanont and Tang, (2012), a biologic asset’s Pharmacokinetics should be well-behaved such that its properties should be predictable in the long-term. With this property, high prospects biologic assets’ success are expected as there will be low-attrition rates. However, it should be noted that the well-behaved property requirement for biologic assets has been challenged. The reason for this is that the property is characterized by on-target specificity while off-target monoclonal antibodies have been found to interact, placing a biologic molecule’s efficacy at stake (Bumbaca et al., 2011). Such off-target antibodies are known to result in rapid clearance of the on-target specific biologic molecule, hence limiting its efficacy by enhancing poor target tissue biodistribution. In
addition, non-specific interactions of antibodies with biologic molecules have a great potential of not enhancing the much needed specific cross-reactivity that is also very rare (Wang et al., 2011). Therefore, it is highly possible that the use of such biologics may result in the subjects developing some clinical conditions that the researchers did not expect. This is the reason why the biologic molecule ought to be well-behaved.

When a biologic asset exhibits poor Pharmacokinetics, they are terminated at some point during their development and designation as a fast to FIH. The reason for this is that such poor properties may have safety issues (Dostalek, Pruksaritanont, & Kelley, 2017). As much as the biologics may be recording low attrition rates during the clinical development stage, the good record may not be replicated during the subsequent stages of development. Consequently, the biologic molecule shall have violated the well-behaved requirement. However, it should be noted that preclinical development tests are usually carried out on animals; hence they are limited to in vivo Pharmacokinetics studies. Laboratory animals such as dogs, rats, and monkeys are used (Wang & Pruksaritanont, 2010). Therefore, an empirical allometric scaling approach has to be done to make the necessary prediction of human Pharmacokinetics (Li et al., 2016). This prediction is usually carried out without a mechanistic understanding or consideration of the molecular properties (Vugmeyster et al, 2011).

In consideration of the above-mentioned factors, a biologic molecule ought to possess well-behaved properties for a successful designation as a fast to FIH. It is required that a biologic asset to be designated as a fast to FIH behaves just like a drug because it is to be used as the treatment for cancer. The reason for the well-behaved requirement for a biologic molecule is that it is required to initiate a drug-like response in the target cell or protein (Wiley, 2016). There are some biologics meant to treat cancer, which is designed to interact with specific immune system cells.

It has been established that the existent literature majorly focuses on the processes and principles used in designating biologics assets as a Fast to FIH (First-in-Human) program. However, the assumptions that are made when developing biologics as a fast to FIH have not been explored sufficiently previously. In addition, the literature on utilization of the processes of high and low-risk activities by pharmaceutical companies using Fast to First-In-Human (FIH) model and their successful implementation and subsequent readiness for regulatory submission of the clinical trial application (CTA) is scarce. Therefore, it is implicit that insufficient research has been carried on the area. The proposed study will fill the existent gap in literature and scientific applications by exploring and ascertaining the principles, assumptions, and processes utilized in designating biologics assets as a Fast to FIH (First-in-Human) program, as well as, streamline the processes of high and low-risk activities using Fast to First-In-Human (FIH) model and their successful implementation and subsequent readiness for regulatory submission of the clinical trial application (CTA).

Methods

The proposed research will utilize a qualitative survey study approach. Therefore, a survey will be carried out on at least five leading pharmaceutical companies (Company A, B, C, D and E), using mailed questionnaires. Questionnaires will be used to collect information pertaining to the principles, assumptions, and processes utilized in designating biologics assets as a Fast to FIH (First-in-Human) program, as well as, streamline the processes of high and low-risk activities using Fast to First-In-Human (FIH) model and their successful implementation and subsequent readiness for regulatory submission of the clinical trial application (CTA). The questionnaires will be directed to the members of the research and development departments as well as analytics of the selected pharmaceutical companies. A qualitative approach has been chosen as an ideal methodology because the information required to answer the main research question and test the hypotheses cannot be quantified (Rahman, 2016; Tolley, Ulin, & Robinson, 2013). Therefore, qualitative information regarding the phenomenon under study will be collected.

Research design

The proposed research will utilize a qualitative survey study approach. Therefore, a survey will be carried out on 5 leading pharmaceutical companies, Amgen Inc. Eli Lilly and Company, AstraZeneca, Johnson & Johnson, and Merck & Company Inc., using mailed questionnaires. Questionnaires will be
used to collect information pertaining to the principles, assumptions, and processes utilized in designating biologics assets as a Fast to FIH (First-in-Human) program, as well as, streamline the processes of high and low-risk activities using Fast to First-In-Human (FIH) model and their successful implementation and subsequent readiness for regulatory submission of the clinical trial application (CTA). The questionnaires will be directed to the members of the research and development departments of the two pharmaceutical companies. A qualitative approach has been chosen as an ideal methodology because the information required to answer the main research question and test the hypotheses cannot be quantified (Rahman, 2016; Tolley, Ulin, & Robinson, 2013). Therefore, qualitative information regarding the phenomenon under study will be collected.

The proposed study will focus on the principles, assumptions, and processes utilized in designating biologics assets as a Fast to FIH (First-in-Human) program, as well as, streamline the processes of high and low-risk activities using Fast to First-In-Human (FIH) model and their successful implementation and subsequent readiness for regulatory submission of the CTA. The study will adopt an empirical research, whereby a total of 40 members will be interviewed, which means that the researcher will interview 20 informants from each of the two companies. The targeted respondents will be researchers from the two companies’ research and development departments. Specifically, the informants will be required to respond to the questionnaires, which will be mailed to them electronically. It is imperative to carry out empirical research as proposed in this study because contextual information regarding the phenomenon will be acquired. In addition, the researcher will gather knowledge on the collective experiences of various organizations in the development of biologics to confirm the theoretical concepts that have been put forward. Consequently, appropriate responses to the dynamics of the phenomenon will be forged while the provision of contextual differences will be possible. In addition, empirical research may help in advancing knowledge on the basis of what is known regarding the development of biologics.

Target population

According to Subong (2005), a population is defined as the set of individuals, objects, or data from where a statistical sample can be drawn. Population is the entire group of individuals, events or objects having a common observable characteristic. A population can be the total sum of collected units from which the researcher draws conclusions of the study (Jansen, 2010). Separately, it should be noted that the target population is the group of people to whom the researcher wants the study findings to apply. Therefore, a study that seeks to find out the effects of a certain disease on a community will focus on a section of the members of that region as the target population. For instance, the study may target on people who have been affected by the disease before, but have survived. It may also target their family members and relatives, as well as, children. Another example is when a company seeks to launch a new product targeting senior citizens as they would be the appropriate users. The researcher is required to analyze the target population, which in this case is the senior citizens’ population. The reason for this is that the analysis of the target population provides insights that can allow the organization to make valid inferences regarding the type of advertisements and campaigns that should be executed to different senior citizens in consideration of their income levels and attitudes.

The study population is closely related to the target population. However, the study population of a research is defined as the objects or people who meet the researcher’s operational definition of the target population. The target population is a broad grouping of the objects to be studied, which is later narrowed down to study population and then it is narrowed further to arrive at the research sample. The research sample forms the members or objects of the study population from which the researcher collects information.

According to Lavrakas (2008), the target population for a survey refers to the whole set of units for which the survey information is used to make inferences. Therefore, the target population denotes the units that the results of the survey are meant to generalize. A survey is designed through a series of steps, the first of which is the establishment of the research objectives. The objectives are crucial in determining the kind of information required and the source from which such information should be obtained. They also determine how the information is analyzed and presented to make reliable and
valid inferences. Consequently, the second step involves the definition of the target population. It follows that specific definition of the target population is crucial as it is the determining factor of whether the sampled objects or case can be used in survey as eligible or ineligible elements. It is also imperative that the geographic characteristics and other characteristics of the target population that may be temporary should be described or portrayed appropriately. Additionally, the researcher should specify the type of units that will be used in the target population. The researcher should ensure that the target population is made up of units that can be used to obtain reliable information easily (Lavrakas, 2008). Therefore, the target population may be restricted in some instances so that those elements that are difficult or impossible to obtain information from them are excluded. For instance, if the target population includes difficult to interview people, then the researcher will have to technically leave out such people out and focus only on those that are easier to interview.

The target population should be a population of elements that are experimentally accessible. In this case, an experimentally accessible population refers to the population that a researcher can measure practically and obtain tangible results. However, it should be noted that sometimes researchers face constraints that bar them from accessing and interviewing the ideal target population. An ideal example of the constraints that a researcher faces is budgetary limitations. When there are budgetary constraints, the researcher can only interview a limited number of people from the target population. In such cases, the experimentally accessible population differs significantly from the target population because the former is much smaller than the latter. In ideal cases, the two are supposed to be almost equal in order to achieve the threshold of representativeness. Separately, some physical factors can limit the researcher from interviewing a significant number of people from the target population. Consequently, the researcher ends up interviewing a small number of people because he or she is forced to select a smaller group for study. This applies when the researcher is required to interview a population and finds out that it is not feasible to interview every member of the population because the members could be dispersed geographically. Therefore, the researcher settles on a smaller population or sample population that he or she interviews and generalizes the results for the entire population. However, when the experimentally accessible population is not large enough, it does not provide a significant representation of the entire target population (Mack, 2017). Therefore, the study results cannot be generalized for the entire population and offer valid and reliable inferences. The researcher should ensure that he or she chooses an ideal target population for the study sample so that to carry out investigations on a significant representative of the entire population.

In this case, the researcher will avoid interviewing just a few participants for the study because that will jeopardize the reliability and validity of the results and inferences. In addition, the researcher will choose the target population to study appropriately by using the most reliable statistical methods to ensure that the results obtained will be valid for understanding the target population at large. The number of participants to be interviewed will be increased significantly as the researcher will avoid focusing only on the conveniently located informants. Consequently, the chances of obtaining biased results that that are not true for target population will be minimized or eliminated. Alternatively, the researcher will utilize the possibility of selecting a representative sample from the experimentally accessible population. This means that the researcher will seek to base the investigation on a large enough group of informants. Additionally, the researcher will utilize appropriate and purposive selection methods to enhance the confidence the results of the research will be valid for the whole target population.

The target population of this study will be leading pharmaceutical companies, especially global companies. The researcher targets leading pharmaceutical companies because unlike the other smaller pharmaceutical companies, they engage in the manufacture of FIH drugs. Therefore, they are mostly likely to expected to utilize various principles, assumptions, and processes in designating biologics assets as a Fast to FIH (First-in-Human) program, as well as, streamline the processes of high and low-risk activities using Fast to First-In-Human (FIH) models. Moreover, they are always keen to enhance the successful implementation process to facilitate subsequent readiness for regulatory submission of the clinical trial application (CTA). Therefore, the study targets the leading pharmaceutical companies from the United States. It is not only the companies that the study will
target, but also its key employees and management teams, which are most likely to have the information that is required.

**Sampling frame**

A sampling frame is the list of individuals or events, source material or device from which a sample is drawn (Lavallée, 2007). It comprises of a list of all those within a population who can be sampled, and may include individuals, households, companies or institutions. According to Turner (2003), a sampling frame is the set of materials that act as an informational source from which the researcher selects the sample for information gathering purposes. It follows that the sampling frame is the source of the means of particular members of the target population that the researcher can choose so that they can be interviewed during a survey. In general, there may be more than one set of materials available to be selected as the ideal sample for study. This applies to descriptive surveys, like in the case of the proposed research because it entails interviewing of a group of people with similar characteristics ideal for the provision of accurate and relevant information required by the researcher. Moreover, the proposed study will be multi-stage in nature. In this case, the researcher will identify the area frame and come up with a list that is comprised of various organizations to form a list frame. Therefore, the researcher will select the sample, first from the area frame, and then narrow it down to a list frame from which organizations for that and respective informants will be selected.

In the case of this research, the researcher will make various important considerations when choosing the appropriate sampling frame. Particularly, a consideration of the relationship between the research target population and the unit of selection will be made by the researcher. Given that the target population determines the unit of selection, the proposed study will consider organizations that manufacture Fast to FIH (First-in-Human) drugs, utilizing high and low-risk processes to enhance the successful implementation and subsequent readiness for regulatory submission of the clinical trial application (CTA). In addition, the unit of selection determines the probability of selection at the last stage. Therefore, the researcher will be guided by these principles while choosing the sampling frame and the sample for study from the target population.

The researcher will ensure that the sampling frame captures, in a statistical sense, the target population by choosing the most representative sample from the target population. The researcher will also ensure that the information to be obtained from the investigation will be as perfect as possible by choosing a complete, accurate, and up-to-date sampling frame. As much as this ideal property may be unattainable in the proposed survey, the researcher will ensure that it meets these requirements by constructing the sampling frame from scratch rather than using an existing one. Consequently, the sample frame will be up-to-date and as accurate as possible, though it may be as complete as may be required.

The researcher will ensure that the sampling frame meets the basic conditions of high-quality by assessing it in terms of how well the frame’s idealized properties are related to the targeted population. Particularly, the researcher will ensure that the rules of selection are duly followed such that every member from the target population will have an equal chance of inclusion just like the rest. The chance of selection in this case will be known and non-zero. Therefore, the researcher will ensure that these conditions are met so that the sampling frame’s quality may be objectively assessed.

However, it is noted that the sampling frame may not be as complete with respect to the target population as theory indicates because an ideal frame should have all of its members or the entire the universe covered. Given that these conditions will not be met in absolute terms in the case of this study, the proposed sampling frame may not be complete. However, the researcher will ensure that the sampling frame coverage is as wide as possible to facilitate its suitability for study. The researcher will also ensure that the frame meets the basic requirements, and if it does not meet them, then a determination as to whether it can be repaired or developed further to make it suitable will be made. For instance, if a survey seeks to investigate a certain aspect among the children born in medical facilities, then the sampling frame will not include those children born at home and at other places, apart from medical facilities. The reason for this is that such a survey seeks to investigate an aspect among children born in medical facilities only as the sampling unit. In such a case, there will be significant numbers of the target population that will have a zero chance of inclusion in the sample,
and the condition for a probability sample is will be violated. Similarly, in this case, the researcher seeks to investigate pharmaceutical organizations that usually produce FIH drugs. This means that other pharmaceutical companies that do not manufacture FIH drugs will not be included in the study, and this would result in the violation of the probability sampling rule. However, the results will still be accurate and representative enough because the researcher’s hypotheses shall have been tested using the most valid and reliable information from organizations and informants with the required information. It would not be expected that accurate and reliable information regarding the principles, assumptions, and processes established to designate biologics assets as a Fast to FIH (First-in-Human) program if organizations that do not manufacture such drugs are surveyed. Similarly, the research will not be able to establish whether there have been any significant changes in the associated core concepts for the utilization of low and high-risk activities, timelines, and functional level expectations in the designation of biologics assets as a Fast to FIH by interviewing employees from other pharmaceuticals that do not manufacture FIH drugs. Therefore, the researcher will not present biased results.

The researcher will ensure that the accuracy of the findings is enhanced by including each member of the selected member form the target population in the research only once. In this case, the researcher seeks to investigate, through the proposed study, the leading pharmaceutical companies that manufacture FIH drugs, particularly those that utilize low and high-risk activities. Therefore, it would be erroneous to include pharmaceutical companies that do not fall under the category of “leading” in terms of performance, those that do not manufacture FIH drugs, and those that do not utilize low and high-risk activities in the study. It would also be erroneous to include a company more than once in the study (Turner, 2003).

It will be ensured that the proposed study addresses the issue of coverage error appropriately. According to Turner (2003), coverage error is the items of study or people that are excluded from a study sample. In typical cases, sampling statistics are calculated assuming there is no coverage error. When some population subjects are left out systematically from the sampling frame, then it is apparent that the sampling statistics of such a research will not have to account for the cover coverage error. Examples of coverage error include a study that involves telephone users as the main subjects under investigation, where the people without telephones are excluded systematically. In addition, those who have cell phones will also be left out because they do not meet the basic requirement of owning a telephone as a cell phone is quite different from a telephone. On a separate account, a study that seeks to investigate internet users will always leave out non-internet users.

Researcher bias emanates from the nature with which the research objects are under-covered, especially when the magnitude between the covered and the uncovered subjects. For instance, a study on telephone users may not differ in terms of votes if cell phones were used. However, there is a huge possibility that they may different opinions and attitudes towards technology. In this case, the researcher will leave out pharmaceutical companies that do not manufacture FIH drugs. However, this will not affect the results in terms of bias because such organizations that will not be included will not have any significant opinion regarding the research topic.

The proposed study will use a sampling frame of organizations that manufacture FIH drugs, particularly leading pharmaceutical companies as a tool that will enable the researcher to objectively select a sample of units from the population of all units. In this case, it will be considered whether the sample results will lead to generalizable conclusions and whether the proposed sampling plan will be possible within time and budget limitations. In addition, the researcher will consider whether the sampling procedure will be practically feasible. Finally, the researcher will investigate whether the proposed sampling scheme will provide results that will address survey objectives with appropriate measures of precision. It should be noted that the quality of the sampling frame usually affects the quality of the sample. The researcher will ensure that there is adequate information on the frame available so that sampling, data collection, weighting, and non-response bias analyses can be conducted. Therefore, the researcher will ensure that the sampling frame of this proposed study up to date and includes only one record for each member of the target population.

As indicated, the sampling frame for the study will be derived from a group of the leading companies in the pharmaceuticals industry in the United States. There are numerous companies in the
US pharmaceutical industry. However, the researcher will concentrate on the top 10 companies and then choose 5 of them randomly. Therefore, the sampling frame will consist of 17 companies as indicated in Appendix 1. The companies include; Amgen Inc., Eli Lilly and Company, Johnson & Johnson, Pfizer Inc., Watson Pharmaceuticals Inc., Merck & Company Inc., AstraZeneca, Novo Nordisk, Sanofi, Bristol-Myers Squibb, AbbVie, GlaxoSmithKline, Gilead Sciences, Roche, Abbott Laboratories, Biogen Idec, and Calgine Corporation.

### Table 1. Sampling frame

<table>
<thead>
<tr>
<th>Company Name</th>
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<tr>
<td>1 Amgen Inc.</td>
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<td>2 Johnson &amp; Johnson</td>
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<tr>
<td>3 Eli Lilly and Company</td>
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<tr>
<td>4 Pfizer Inc.</td>
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<tr>
<td>5 Watson Pharmaceuticals Inc.</td>
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<tr>
<td>6 Merck &amp; Company Inc.</td>
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<td>7 AstraZeneca</td>
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<td>8 Novo Nordisk</td>
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<td>10 Bristol-Myers Squibb</td>
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<td>12 Glaxo SmithKline</td>
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<td>13 Gilead Sciences</td>
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<td>14 Roche</td>
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<tr>
<td>15 Abbott Laboratories</td>
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<tr>
<td>16 Biogen Idec</td>
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<tr>
<td>17 Calgine Corporation</td>
</tr>
</tbody>
</table>

### Sampling techniques

Non-probability sampling will be utilized in the proposed study. The reason for this is that not all the organizational members of the two companies will have a chance of being selected as informants. Instead, the researcher will purposively select informants from among the researchers of the two companies’ research and development departments. The reason for choosing purposive sampling is that it will enhance the collection of phenomenal information from people who have experience (Adler & Clark, 2014). Therefore, precise and accurate information will be collected through this kind of sampling. Purposive sampling is advantageous because it focuses on individuals who have desired characteristics to provide relevant information for answering the research question (Macnee & McCabe, 2008).

The researcher will ensure that all checks regarding the sample and its quality are carried out. For instance, the researcher will calculate the sample error, which refers to the description of describes the variability of a sample statistic across multiple hypothetical samples that could be drawn. It is calculated on samples and is based on statistical theory. An investigation of the variation between different potential samples of respondents and the entire sample frame will also be done. It should be noted that coverage error and non-response error are not accounted for in sampling statistics. There will be no bias into the study as there will be no coverage error or non-response error because they will be systematic.

### Data collection instruments

The research will use a questionnaire as a data collection instrument for data. The use of a questionnaire will help the researcher to obtain the best information from the respondents because it can be tailored to suit the information requirements of the research. For instance, the questionnaires will have both closed and open-ended questions because the researcher will want respondents to provide specific information while allowing them to provide their own perceptions. Closed questions will require respondents to choose one answer to the question from a number of provided options
while open-ended questions will provide respondents with an opportunity of suggesting their views. Therefore, open ended questions are ideal for gathering qualitative information. This will result into precise and rich information for the researcher because questionnaires help in the collection of straight forward information. The researcher will issue self-administered questionnaires to respondents will fill the questionnaire themselves, in the absence of the researcher. This will allow anonymity, thus allowing respondents to provide honest answers (Mitchell & Jolley, 2010). Therefore, a questionnaire will be an ideal instrument because it will enhance flexibility in formulating questions, which will help in gathering relevant information for answering the research questions. Similarly, in this case, the questionnaires will be the main data collection interviews that will be used in this research. The reason for this is that they are convenient for data collection (Creswell, 2014). The researcher will design appropriate questionnaires, comprising of questions that will seek to gather accurate information for answering the main research question and testing the hypotheses. The questionnaires will be used in a pilot study, which will entail surveys of ten randomly selected staff members from each of the two companies. The pilot test questionnaires will be used to test the validity and reliability of the data collection instrument. Therefore, questionnaires will be evaluated whether they accurately seek to gather the intended information, depending on the pilot study responses. It is expected that any adjustments to the questionnaires will be made on the basis of the pilot respondents' suggestions. Questionnaires will be sent to the respondents through email.

Results

Results are upon further discussion. It is expected that upon the completion of the proposed study, insightful and empirical information about a new the existing phenomenon regarding biologics development shall have been obtained. In this case, it is expected that the research will establish there have been newer principles, assumptions, and processes established to designate biologics assets as a Fast to FIH (First-in-Human) program. Similarly, the research expects to establish whether there have been any significant changes in the associated core concepts for the utilization of low and high-risk activities, timelines, and functional level expectations in the designation of biologics assets as a Fast to FIH. This will be further discussed in next available article to support the applied methodology.

Discussion

The above study contributes to scientific knowledge because its findings can guide drug developers, especially biotechnology and pharmaceutical companies that develop biologics for oncology treatment and immuno-oncology purposes on the principles, processes, and assumptions to make when designating such assets as a fast to FIH. Consequently, society will benefit from the study’s findings because it is expected that with the right knowledge, scientists and biologic developers will come up with better health sustaining solutions. In addition, society will benefit from the availability of cancer-treating biologics developed by relevant companies. It is expected that biologic developing companies will utilize this study's findings to improve their approaches in new drug development. Consequently, there will be a variety of effective drugs for the treatment of cancer available at affordable costs, which will save society from the economic burden of taking care and paying for the treatment of the sick.

Conclusion

Further evaluation will be discussed to focus on established already principles, assumptions and processes that are most utilized by current pharmaceutical companies. First-in-human studies are included phase 1 trials where the initial human exposure is initiated to the investigational new drug. Clinical phase 1 is critical and will be substantially evaluated under new assumptions since it affirms if a compound’s mechanisms of action in humans and its development can result in a potentially new drug entity.
References


Differentially... 


A Parallel Group Randomised Control Study to Investigate the Efficacy of Weekly Motivational Text Messages on Adherence among Clients on Anti-Retroviral Therapy- Kadoma Mobile Phone Study (KÂMPS) 2016-17

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Abstract

Background: Anti-retroviral therapy was introduced in 1997 to manage people living with the Human Immune-deficiency virus (HIV). The success of treatment is dependent on optimal levels of 95% or more adherence. A 2015 study conducted at the Rimuka Integrated HIV and TB Centre, revealed a suboptimal adherence rate of 87% by self-report and 65% by pill counts. We set out to investigate the efficacy of cell phone short message service on adherence among clients on ART at the center.

Methodology: KÂMPS was a parallel design randomized controlled trial of HIV clients receiving ART at Kadoma (Zimbabwe). Respondents were randomised 1:1. Respondents in the intervention group received a weekly motivational SMS in addition to standard HIV care whilst those in the non-intervention arm received standard care alone. The primary outcome was adherence measured using a composite scale. Secondary outcomes were weight, CD4+; and, viral load, measured at baseline and 26 weeks. The primary analysis was by intention to treat. The trial was registered PACT20161001858240.

Results: Of the 552 assessed respondents, 470 were eligible and were randomized into the study. However, analysis was done for 449 respondents. At 26 weeks, 180 (76.9%) respondents in the intervention group were considered adherent compared to 127(59%) in the non-intervention group (p=0.65). The mean CD4+ cell count at six months was 554 cells mm⁻³ among those in the non-intervention group compared to 619 cells per mm⁻³ among those in the intervention group. Mean viral load was 392 copies ml⁻¹ of blood in the intervention group compared to 2859 copies ml⁻¹ in the non-intervention arm. Among those in the intervention group 140(60%) had viral suppression compared to 93 (43%) in the non-intervention arm.

Conclusion: We conclude that weekly motivational short message influenced adherence and ultimately CD4+ cell count, viral loads and viral suppression.

Keywords: Adherence, ART, HIV, Kadoma, mHealth, Zimbabwe.

Introduction

Antiretroviral (ARV) medications are treatments for people living with the Human Immunodeficiency Virus (HIV) whose aim is to drive viral load below the current detection limit. ARV medications were not available for treatment of HIV/AIDS complications prior to approval by Food and Drug Administration 1986. They became publicly available in 1997 after introduction of two drugs Zidovudine and Lamivudine (Diederich W.E. & Stember H. 2015).

Successful treatment with ARVs reduces the destruction of cluster of differentiation 4+ (CD4+) cells, immune suppression, HIV transmission and, slows disease progression. This can be seen in rising CD4+ cell counts, undetectable viral loads and clinical improvement. As viral load assays are expensive in resource limited settings, CD4+ counts and clinical physical examination are the main tools for monitoring treatment outcomes (Hammer S., et.al. 1997).

Unfortunately, there are several problems associated with anti-retroviral therapy (ART). These include the development of drug resistance, the difficulties of maintaining compliance, long-term adherence and drug-related toxicities. All these may lead to virological failure. Virological failure in
turn leads to immunological failure, clinical progression of HIV and ultimately death (Mocroft A. et.al. and Department of Health and Human Services 2017). Adherence to ART is, critical to the survival of HIV infected individuals (The PLATO Collaboration 2004). The optimal level of adherence required for ARVs medication to work effectively is 95%. (Paterson David L., et.al. and Reiter G., et.al. 2000). Anything less than this optimal level of compliance leads to rapid development of viral resistance and hence to treatment failure. According to Lima et. al. (2000), for a five percent reduction in adherence, there is a fivefold increase in virological indices. Therefore, successful HIV treatment requires strict adherence to medication.

Adherence is defined as: ‘The extent to which the patient’s behaviour matches agreed recommendations from the prescriber.’ (Horne R. et.al. 2006). In HIV infection, adherence to ARV has been strongly correlated with viral suppression and results in improved clinical presentation and health outcomes (Bangsberg D. 2006). Non-adherence is associated with development of HIV drug resistance whose risk is highest in individuals with incomplete adherence (e.g. 80%-90%). The link between adherence and development of resistance depends on potency and pharmacokinetics of the regimen combinations. The phenomenon of non-adherence has been linked to limited future HIV treatment options and combinations causing HIV drug resistance and increases risk of HIV transmission (Mills Edward J. et.al 2006 and Palella FJ. et.al 1998).

Although adherence has emerged as a major determinant of the success associated with ART, it is not the only determinant of ART success or failure (Braitstein P. 2006). Other determinants of ART failure or success include genetic differences in drug metabolism, severe baseline immune suppression, prior drug resistance and concurrent opportunistic infection (Reiter G., et.al. 2000). Adherence to ART however, remains, one of the few alterable factors determining outcomes for patients with HIV. ART adherence is the second strongest predictor of progression to AIDS and deaths. The first predictor being CD4+ count (Ickovics J.R. & Meade C.S. 2002).

It is imperative that patients on ART be supported to ensure treatment success. Of late mobile health technology (mHealth) has emerged as a tool to support health care delivery including improving adherence (Ickovics JR, & Meade CS (2002) and Labrique Alain B. et.al. 2013). Several studies, among them clinical trials have been conducted to evaluate the utility of mHealth and the use of reminders, to promote adherence to ART (Mbuagbaw L et.al. (2011); Pop-Eleches C. et.al. (2011); Kelly J. et. al.; Lester R. et.al. (2010); Dongsheng H., et.al. (2013); da Costa, et. al. (2012) and Hardy et. al. (2011). However, there are still many gaps and the quality of some of the trials leaves a lot to be desired.

A 2015 study on adherence to ART among clients seeking HIV care at Rimuka Integrated HIV and TB Centre (RIHTC) revealed an adherence rate of 87%. This was based on self-reports. However, pill counts at the health center among a subset of respondents, revealed that the adherence was 65% (Muringazuva C et.al. 2015). This is a cause of concern since ART adherence should be above 95% to have virological or public health impacts (Paterson David L., et.al. and Reiter G., et.al. 2000). It is against this background that we set out to investigate novel ways to improve adherence among clients on ART so that the benefits of treatment are maximized.

We hypothesized that clients on ART who receive a weekly motivational SMS in addition to standard HIV care at RIHTC are more likely to be adherent to ART than those receiving standard HIV care alone. We set out to investigate the efficacy of cellphone short message reminders on adherence to antiretroviral therapy among clients seeking HIV care at the Centre.

Methods

We report here an overview of the methods. Details of the study protocol are published elsewhere (Chirundu, D. et.al. 2017).

Study design

This was a parallel design randomized controlled trial (RCT) to investigate the efficacy of weekly motivational short message service (SMS) on adherence to ART.
Trial setting

Rimuka Integrated HIV and TB Centre (RIHTC) was the trial setting. The facility is operated by Kadoma City Health department in Zimbabwe. Other facilities providing health care in the city include six municipal clinics, eight private practitioners, two private hospitals and one government referral centre. All these except one offer various HIV services. The HIV prevalence in the town was estimated to be 14% in 2016. The total number of clients on ART who were being managed by Kadoma City Health was 8225 in 2016.

Study population and study unit

The study population were HIV clients enrolled for care at RIHTC. The study unit was an individual client on the RIHTC register.

Eligibility

We included in the study any person, registered on the RIHTC ART register, who has been on ART for at least 4 weeks, owned a cell phone, and, was willing to receive SMS reminders for adherence. In addition, they should not have been planning to move out of Kadoma permanently during the study period and gave written informed consent to participant in the study. Those below 18 years and the severely sick were excluded.

Intervention

In the intervention group, participants received a motivational SMS reminder on a weekly basis for the duration of the trial. This was in addition to the standard HIV care afforded to clients. Messages were developed from message preference data collected at baseline. The message had a motivational and a reminder component. The message also included a phone number on which the client could raise any issues to do with treatment. The message content varied from week to week and was contemporary. An example of a message would be: “We are there for you; please remember to take your medication for any concerns contact +263 773 xxx xxx.” The messages made no mention of the words HIV or ART. In the non-intervention group, the participants received standard care as prescribed by the Zimbabwe ART guidelines. This comprised of CD4+ cell count, adherence classes, and, follow up (Ministry of Health. Zimbabwe 2016).

Primary outcome

The primary outcome was adherence to ART measured on a composite scale comprising of self-reported adherence, visual analogue scale (VAS), pill identification test (PIT) and pill counts. Participants were designated adherent to ART if they were considered adherent on all the four components. The method was piloted in South Africa (Steel G. et. al 2007).

Secondary outcomes

Secondary Outcome measures were weight, body mass index (BMI), CD4+ cell count, and viral load. The outcomes were measured at baseline and 26 weeks.

Sample size

The sample size calculated using Open Epi Version 3.03a™ was 306 assuming a 1:1 allocation ratio. The study had 80% power to yield a statistically significant result using a chi-squared test at alpha = 0.05/2. This calculation assumed a 10% improvement in ‘adherence’ over a baseline of 85% determined from the pilot by Muringaziva et.al (2015); rates which we felt were most representative of data available for Kadoma. Assuming attrition rates of approximately 10% we intended to enrol at least 332 participants for randomization.

Randomization and allocation concealment

A list of all clients in the RIHTC register who satisfied the inclusion criteria was compiled. The list was sequentially numbered by a data capture clerk. This was our sampling frame. A health information clerk generated random numbers using the Microsoft Excel™ random number generator. The data
capture clerk and the health information clerk handed over the sampling frame and the random numbers to the Clinic Manager. The Clinic Manager then compiled a list of the clients whose numbers on the ART registered corresponded with the randomly generated numbers. These were invited to participate in the trial. A recruiting nurse then did the group allocations. Allocation concealment among those who gave consent was done as described by Doig et al. (2005). There was no restriction or blocking.

Data collection and analysis

Data were collected using a pretested interviewer administered questionnaire at baseline, and six months. Questionnaires were administered by data collectors who were fluent in the languages of the respondents. Adherence was assessed at each visit by enquiring about missed doses in the previous 30 days and calculating adherence according to pill counts. A weekly log was used to record the weekly SMS messages and any responses. A study register was kept for monitoring follow up.

The analysis and reporting of the results complied with the CONSORT, Consort e-health and SPIRIT 2013 guidelines (Gunther Eysenbach, 2011 and Chan An-Wen 2013). The process of patient selection and flow throughout the study was summarized using a flow-diagram. The intention-to-treat principle was used to analyse all outcomes (Lewis' J.A. & Machin D. I (1993) & Montori V and Gordon H. Guyatt: 1993). We used the t-test for comparing groups on continuous outcomes and the chi-squared test for binary outcomes. All statistical tests were performed using two-sided tests at the 0.05 level of significance.

Ethical considerations

Written informed consent was obtained from all participants. The protocol was registered by the South African Medical Research Council (PACT20161001858240). Participants were free to withdraw from the study at anytime.

Results

Consort diagram

Five hundred and fifty-two (552) respondents were assessed on their eligibility to participate in the study. Five hundred and twelve (92.7%) among those assessed owned a cell phone. Sixteen (3.06%) intended to move out of Kadoma. The consort diagram is presented in figure 1.

Demographic characteristics of respondents

Whilst we assessed 522 potential respondents for the study, analysis was done on 449 respondents. Among these 320 (71.27%) were females and 129 (28.73%) were males. The median age of the respondents was 43 years (Q1=36; Q3=49). The median number of months since HIV diagnosis was 59 (Q1=34; Q3=87). The median number of months on since ART initiation to baseline study was 45 (Q1=29; Q3=72). The demographic characteristics of the respondents stratified by the intervention arm are presented in table 1. The only variable in which there was a significant difference in the proportions among those in the non-intervention and intervention groups was being married (p=0.0001).

Patterns of cell phone usage

Among the 449 (100%) respondents for which analysis was done, 415 (93.63%) could read or send an SMS. The preferred method of communication on a cell phone was text 333 (75.17%), followed by voice 82 (18.51%). The preferred language for SMS communication was Shona 310 (72.09%), followed by English 115 (26.79%). Eighty-two (18.47%) of the respondents reported losing or having the phone damaged 12 months prior to the study. During the study 52 (11.76%), reported losing or having phone damaged. The median duration without a handset was 4 weeks (Q1=2; Q3=8). Four hundred and thirty-four respondents (97.75%) thought a text message could be useful for improving adherence to ART. However, 430 (97.07%) were willing to be reminded by SMS to take ART medication. Cell phone usage patterns stratified by intervention arm are presented in table 2.

There were no differences in the proportions of respondents across intervention arms on most of the variables we assessed on cell phone usage (p>0.05). The only significant difference was noted in the proportions of respondents who indicated that a text message received on their phone was somewhat
likely to be seen by others \((p=0.03)\). On the preferred message analysis none of the respondents preferred the word “HIV” or “ART” to appear in the message. However, 196 (43.65%) were comfortable with the word “medication” appearing in the message.

**Adherence rate by various measures**

At baseline 208 (96.74%) among those in the non-intervention arm reported an adherence rate greater than 95% by self-report. This increased to 210 (97.67%) after six months. Adherence measured by pill count had the lowest proportion of respondents with more than 95% adherence, 146 (67.90%), at baseline and this proportion decreased to 138 (65%) at the end of six months. Composite adherence measure had 131 (60.93%) respondents being adherent, and this decreased to 127 (59%) respondents at the end of 6 months. However, all these changes in proportion were not statistically significant \((p>0.05)\).

Among those in the intervention group, 223(95.37%) of the respondents had more than 95% adherence measured by the self-report method at baseline. Adherence measurement by pill count had the lowest proportion of respondents with more than 95% adherence, at 159 (67.90%) of the respondents. After six months the proportion of respondents in the intervention arm with more that 95% adherence by self-report was 229 (97.96%) whilst the proportion of those with more than 95% adherence by pill count had moved to 189 (80.76%). All the changes in proportions were statistically significant \((p<0.05)\). The changes in adherence by various adherence measure in the experimental arms are presented in Table 3.

The proportion of respondents who were adherent by various measures stratified by intervention arm are presented in Figure 2. At baseline there were no significant differences in the proportions of adherent respondents by self-report \((p=0.72)\), visual analogue scales \((p=0.71)\). However, there were significant different in the proportion of adherent respondents by pill identification test \((p=0.02)\). One hundred and thirty-nine (59%) of the respondents in the intervention arm were adherent by composite measure compared to 131 (60.93%) in the non-intervention arm. However, the difference in the proportions were not statistically significant \((p=0.65)\).

After six months there were changes in proportions of respondents across intervention arms as measured by self-report 229 (97.96%) in intervention arm and 210 (96.74%) in the non-intervention arm \((p=0.48)\). Significant differences across arms were noted in pill identification (228/234 vs 164/215) \((p<0.05)\); pill count (189/234 vs. 138/215) \((p<0.05)\) as well as the composite measure (180/234 vs 127/215) \((p=0.05)\). The changes in proportions of adherent clients at baseline and 6 months across intervention arms are presented in figure 3.

**Over adherence**

At baseline we found 39 (7.95%) respondents with adherence rates above 100%. Fourteen (35.89%) were in the non-intervention group and 25 (64.10) we in the intervention group. The difference in the proportions was statistically significant \((p=0.01)\). At six months the number of respondents with above 100% adherence had decreased to 26 (5.53%). Ten (38.46%) were in the intervention arm and 16(61.53%) were in the non-intervention arms. However, the difference in the proportions was not statistical significant \((p=0.09)\)

**Impact of SMS on health outcomes**

The changes in health outcomes among the respondents in the experimental arms are presented in table 4. Mean CD4+ cell count among those in the non-intervention group decreased from a mean of 593 cells per mm³ at baseline to 554 cells per mm³ at six months. The decrease was statistically significant \((p=0.002)\). Mean viral load also decreased from 4011 copies ml⁻¹ to 2859 copies ml⁻¹. Viral suppression was reported by 93(43%) of the respondents in the non-intervention group. However, the decrease though good was not statistically significant \((p=0.25)\). There was however, a statistically significant decrease in the prevalence of opportunistic infections from 186 (86.51%) prior to the study to 10 (4.65%) during the six months we carried out the study \((p<0.05)\).

Among the respondents in the intervention group the mean CD4+ cell count was 556 cells per mm³ at base line and after six months intervention it increased to 619 cells per mm³. At baseline the means
viral load was 8613 copies ml⁻¹ and it decreased to 392 copies ml⁻¹ at six months (p=0.04). Viral suppression was reported by 140 (60%) in the intervention group. The prevalence of opportunistic infections reported before the baseline study was 168 (71.79%). During the six months intervention the prevalence of opportunistic infections was 6 (2.56%) (p=0.00).

Comparison of CD4+ cell counts at six months

A comparison of CD4+ cells across the intervention arms at 6 months is presented as a box and whisker diagram in figure 4. The means CD4+ cell count in the intervention arm at six months was 619 cells per mm⁻³ whilst that in the non-intervention arm was 554 cells per mm⁻³ (p=0.021).

Comparison of weight changes

The comparison of weight changes across intervention arms at baseline and at 6 months are presented in figure 5. The mean weight for those in the intervention arm increased from 63.01kg to 65.66kg and the increase was statistically significant (p=0.03)¹. On the hand, among those in the non-intervention group the mean weight decreased from 64.32kg to 62.40kg. However, the decrease was not statically significant².

Discussion

This was a Health Centre based RCT study carried out during the period September 2016 through July 2017. The proportions respondents in the intervention arm and those in the non-intervention arm were distributed similarly across most demographic variables, therefore our respondents were comparable.

The proportion of adherent respondents measured on a composite scale significantly increased from baseline to outcome measurement after six months among those in the intervention arm. Notably, the proportion of adherent respondents decreased in the non-intervention arm during the same period. A decrease in the proportion of adherent respondents in the non-intervention group is not uncommon. Similar findings were reported by Pop- Eleshe et al. (2011) in a study carried out in Kenya. As the time on ART increases, patients start feeling better and with this comes treatment fatigue. This is when interventions such as reminders become important to encourage the patients to keep taking their medicines. Counselling to remind them that they are not cured and need to keep taking their medicines in the text messages is also important.

Of concern was that we found over adherent clients by pill count. Adherence rates above 100% are an indication that a client is taking more than the required doses at a time. Alternatively, this may indicate pill dumping. Sharing of medication may also lead to adherence rates above 100% by pill count. Such practise may lead to treatment failure (Reiter G., et.al. 2000 and Balogun M R, et.al.2012). Over adherence may also give results to side effects. If a client is taking more than the required dose, they are more likely to develop the signs of medicine toxicity which may threaten their lives. There is need for further investigation of this phenomenon and its effect on virological outcomes.

We found a higher cell phone ownership coverage of 92.72% among clients on ART at RIHTC. Comparable cell phone coverages were reported in studies carried out by Xiaob et. al. (2013) in China (88%), Kabede et. al. (2015) in Ethiopia (84%), and Person et. al. (2011) in the USA-77%. Most respondents in this study 333 (75.17%) preferred text as routine communication; similar findings were reported Kabede et. al. (2015) who reported a text preference of 70%. We found 369 (82.18%) of the respondents, already using cell phones as reminders for medication. This is similar to 79% usage of cell phones as medicine reminders reported by Tamaryn et. al. (2010) in a study done in South Africa. Such high cell phone coverage presents a unique opportunity for communication with clients outside the health centre setting since health authorities would have ready means to communicate with their clients using cell phones. The high willingness of 97.07% among respondents to receive SMS medical reminders is an additional enabling factor.

Theft or damage to cell phones may derail any SMS interventions, in this study 97 (18%) of respondents reported phone theft or damage in the 12 months preceding the study. This was lower than

---
¹ Used Student T test.
² Used Student T test.
28% reported by Tamaryn et. al. (2010) in South Africa and 51% reported by Kabebe et. al. (2015) in Ethiopia. We found that at six months; the proportion of respondents possessing a phone, changing cell phone numbers, losing or damaging to phones was similar across the experimental arms ($p > 0.05$). It therefore, follows that there was little or no impact on the intervention due to cell phone loss or theft.

We found sharing cell phones common, as such message send can be seen by non-target recipients. This calls for enhanced confidentiality in any SMS intervention. Confidentiality of the SMS reminder is important as some clients have internal stigma. If others see the message, it may result in acted stigma. All these are pertinent issues that HIV control programs should take into consideration at the design stage. Curiosio et. al. (2009) in a study in Peru reported that keeping the medication reminders confidential was the most important concern that many clients on ART expressed. Respondents did not want revealing words like ‘HIV’, ‘antiretroviral’, or any other word related to HIV included in the SMS text. Respondents prefer messages that do not compromise confidentiality.

In the non-intervention group there were significant changes only in mean CD4+ cells count from baseline up to six months. There were no significant changes in mean BMI and viral loads. On the other hand, there were significant decreases in the viral load and increase in CD4+ cell count, as well as increase in BMI from among those in the intervention group at six months. This may be explained in part, by not only being on medication but adhering to the medication as well. This is consistent with the findings of Lester et. al. (2010) in the Weltel Study in Kenya. These findings further emphasise the need to give clients adequate support during their treatment.

We found that 140 (60%) among those in the intervention group had viral suppression compared to 93 (43%) in the non-intervention group ($p=0.004$). A similar rate of suppression was reported by Lester et. al. (2010). Furthermore, we found a significant change in the weight among respondents in the intervention arm. Our result is similar to that reported by Mbuagbaw et. al. (2012) who also reported a significant change in weight among those in the intervention group compared to the control group.

Our study was not without limitations. Rimuka being a predominantly urban high-density area may have different social dynamics to low density areas and rural area. The spatial location may also influence the cell phone coverage. All these issues may limit the generalisability of the results. On the other hand, one may also argue that the high-density resident represents an average Zimbabwean in an urban setting. Another limitation may have been sharing or discussing the motivational messages by those in the intervention group with those in the non-intervention group. This is possible as respondents had opportunity to interact during clinics and support group meetings.

**Conclusion**

It is our conclusion that clients receiving care at Rimuka integrated TB and HIV centre are willing to be reminded to take their medication by means of a cell phone short message and the preferred language is predominantly Shona. However, the content of the message should not contain words like “HIV” or “ART” since the majority share their phones or leave the phones at places where they can be accessed by others.

Adherence among the clients seeking care at Rimuka was moderate based on the composite scale we used. Looking at individual measurement methods, most of the respondents were adherent based on the subjective measurement of the self-report. However, a more realistic measure of adherence was the use of the composite scale combining self-report, visual analogue scale, pill identification and pill count. We recommend that health authorities adopt such a composite scale as is sensitive enough to identify clients who may not be adherent compared to using one method only.

We further conclude that over adherence is a major issue that may threaten ART programs and this needs to be identified early where it occurs. Health authorities need to do hospital based and unannounced pill counts at households to identify and deal with this phenomenon.

Lastly, we conclude that the intervention (weekly motivational) short messages increased adherence (measured using a composite adherence scale) among respondents in this study. There were significant changes in the CD4+cells count, viral profiles. Respondents in the intervention group also had significantly high proportions of viral suppression compared to those in the non-intervention group. We therefore, recommend use of SMS, and, *m*health in HIV care to improve health outcomes.
Notwithstanding the limitations, we believe this work has contributed to knowledge in that it has affirmed that it is better to use a composite method in measuring adherence. Such a measure is more sensitive and can identify clients on ART who are likely to default, and, corrective measures and support could be availed earlier. It has also reaffirmed that weekly motivational short message texts improve adherence to medication and ultimately immunological and virological outcomes. However, large scale implementation of the intervention and its economies of scale need to be further investigated.

In the same vein, we recommend that further studies be done to determine the reproducibility and generalisation of these findings beyond Rimuka Integrated TB and HIV Care setting. We recommend that the intervention be tested in different areas like low-density and rural areas or areas with variability in socio demographic characteristics.

**Figures and tables**

Table 1. Socio demographics of respondents’ kadoma mobile phone study (2016-17)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention Group n=234 (52.12%)</th>
<th>Non-Intervention Group n=215 (47.88%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>166 (70.94%)</td>
<td>154 (71.63%)</td>
<td>0.87</td>
</tr>
<tr>
<td>Male</td>
<td>68(29.06%)</td>
<td>61(28.37%)</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>Median Age (Years)</strong></td>
<td>44(Q1=36: Q3=50)</td>
<td>42(Q1=36: Q3=48)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Marital status:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>166(70.94%)</td>
<td>115(53.74%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Divorces</td>
<td>17(7.26%)</td>
<td>22(10.28%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Separated</td>
<td>14(5.98%)</td>
<td>9(4.18%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Single</td>
<td>15(6.41%)</td>
<td>10(4.65%)</td>
<td>0.42</td>
</tr>
<tr>
<td>Widowed</td>
<td>72(30.77%)</td>
<td>58(26.97%)</td>
<td>0.37</td>
</tr>
<tr>
<td><strong>Religion:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apostolic</td>
<td>57(24.35%)</td>
<td>46(21.40%)</td>
<td>0.45</td>
</tr>
<tr>
<td>Catholic</td>
<td>35(14.95%)</td>
<td>32(14.88%)</td>
<td>0.98</td>
</tr>
<tr>
<td>Muslim</td>
<td>8(3.41%)</td>
<td>4(1.86%)</td>
<td>0.31</td>
</tr>
<tr>
<td>None</td>
<td>6(2.56%)</td>
<td>12(5.58%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Pentecostal</td>
<td>77(32.9%)</td>
<td>81(37.67%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Protestant</td>
<td>37(15.81%)</td>
<td>32(14.88%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Traditional</td>
<td>9(3.84%)</td>
<td>7(3.26%)</td>
<td>0.73</td>
</tr>
<tr>
<td>Other</td>
<td>2(0.85%)</td>
<td>1(0.47%)</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Educational attainment:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary Education</td>
<td>49(20.94%)</td>
<td>42(19.53%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Secondary Education</td>
<td>166(70.94%)</td>
<td>161(74.88%)</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>Employment status:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>4(1.71%)</td>
<td>6(2.79%)</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>Median time since diagnosis (mths)</strong></td>
<td>61.5(Q1=36: Q3=88)</td>
<td>53(Q1=33: Q3=87)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Median duration on ART(mths)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Who owns the house:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Council</td>
<td>21(8.97%)</td>
<td>17(7.90%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Owner</td>
<td>62(26.49%)</td>
<td>46(21.39%)</td>
<td>0.21</td>
</tr>
<tr>
<td>Employer</td>
<td>5(2.14%)</td>
<td>6(2.79%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Variable</td>
<td>Intervention Group (n=234)</td>
<td>Non-Intervention Group (n=215)</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------------------------------------------</td>
<td>-----------------------------</td>
<td>---------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Use Cell phone as medication reminder</td>
<td>194 (82.90%)</td>
<td>175 (81.39%)</td>
<td>0.67</td>
</tr>
<tr>
<td>SMS helpful in adherence to ART</td>
<td>228 (97.43%)</td>
<td>206 (95.81%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Willing to be reminded by SMS to take medication.</td>
<td>228 (97.43%)</td>
<td>202 (93.95%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Preferred SMS language: English</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shona</td>
<td>165 (70.51%)</td>
<td>145 (67.44%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Ndebele</td>
<td>2 (0.85%)</td>
<td>2 (0.93%)</td>
<td>0.92</td>
</tr>
<tr>
<td>Chewa</td>
<td>1 (0.42%)</td>
<td>0 (0.00%)</td>
<td>0.33</td>
</tr>
<tr>
<td>Have alternate number</td>
<td>30 (12.82%)</td>
<td>48 (22.32%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Preferred Communication: Voice</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WhatsApp</td>
<td>15 (6.41%)</td>
<td>13 (6.04%)</td>
<td>0.87</td>
</tr>
<tr>
<td>Text</td>
<td>175 (74.78%)</td>
<td>158 (73.48%)</td>
<td>0.76</td>
</tr>
<tr>
<td>Having phone with you: Always</td>
<td>219 (93.58%)</td>
<td>206 (95.81%)</td>
<td>0.29</td>
</tr>
<tr>
<td>Seldom</td>
<td>6 (2.56%)</td>
<td>1 (0.46%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Sometimes</td>
<td>7 (2.99%)</td>
<td>5 (2.32%)</td>
<td>0.65</td>
</tr>
<tr>
<td>Cell phone damaged or lost in last 12 months</td>
<td>42 (17.94%)</td>
<td>40 (18.60%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Phone replaced with same number</td>
<td>34 (79.7%)</td>
<td>36 (90.00%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Switch off phone during day</td>
<td>19 (8.11%)</td>
<td>22 (10.23%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Stores phone where others have access</td>
<td>167 (71.36%)</td>
<td>150 (69.76%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Shares phones with others</td>
<td>89 (38.03%)</td>
<td>81 (37.67%)</td>
<td>0.93</td>
</tr>
<tr>
<td>Phone has lock code /pattern</td>
<td>37 (15.81%)</td>
<td>42 (19.53%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Able to read or send text</td>
<td>217 (92.73%)</td>
<td>198 (92.09%)</td>
<td>0.79</td>
</tr>
<tr>
<td>Delete text deliberately without reading</td>
<td>18 (7.69%)</td>
<td>17 (7.90%)</td>
<td>0.93</td>
</tr>
<tr>
<td>Text likely to be seen by others:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somewhat likely</td>
<td>18 (7.69%)</td>
<td>30 (13.95%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Somewhat unlikely</td>
<td>7 (2.99%)</td>
<td>3 (1.39%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Very Likely</td>
<td>140 (59.82%)</td>
<td>128 (59.53%)</td>
<td>0.95</td>
</tr>
<tr>
<td>Very Unlikely</td>
<td>67 (28.63%)</td>
<td>51 (23.72%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Use of internet on phone</td>
<td>40 (17.09%)</td>
<td>40 (18.60%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Times do not answer unknown calls</td>
<td>71 (30.34%)</td>
<td>67 (31.15%)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Table 2. Patterns of cell phone use-kadoma mobile phone study (2016-17) 2016

**Preferred Message Content Analysis**

<table>
<thead>
<tr>
<th>Preferred Message Content</th>
<th>Intervention Group (n=234)</th>
<th>Non-Intervention Group (n=215)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reminder</td>
<td>144 (61.53%)</td>
<td>118 (54.88%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Remember</td>
<td>20 (8.54%)</td>
<td>20 (9.30%)</td>
<td>0.78</td>
</tr>
</tbody>
</table>
Table 3. Proportion of adherent respondents by adherence measure and experimental arm- kadoma mobile phone study (2016-17)

<table>
<thead>
<tr>
<th>Adherence Measure</th>
<th>Non-Intervention Group (n=215)</th>
<th>Intervention Group (n=234)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>208 (96.74%)</td>
<td>223 (95.37%)</td>
<td>0.55</td>
<td>0.19</td>
</tr>
<tr>
<td>6 Months</td>
<td>210 (97.67%)</td>
<td>204 (94.88%)</td>
<td>0.54</td>
<td>0.00</td>
</tr>
<tr>
<td>VAS</td>
<td>165 (77.12%)</td>
<td>234(100%)</td>
<td>0.91</td>
<td>0.00</td>
</tr>
<tr>
<td>%</td>
<td>146(67.90%)</td>
<td>197 (84.18%)</td>
<td>0.42</td>
<td>0.0001</td>
</tr>
<tr>
<td>Lost or damaged phone during the study %</td>
<td>138 (65%)</td>
<td>159 (67.9%)</td>
<td>0.67</td>
<td>0.00</td>
</tr>
<tr>
<td>Composite Measure</td>
<td>131 (60.93%)</td>
<td>135 (67.9%)</td>
<td>0.67</td>
<td>0.00</td>
</tr>
<tr>
<td>127 (59%)</td>
<td></td>
<td>180 (76.92%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Virological indices by intervention arm- kadoma mobile phone study (2016-17)

<table>
<thead>
<tr>
<th>Indices</th>
<th>Non-Intervention (n=215)</th>
<th>Intervention Group (n=234)</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CD4+ (per mm⁻³)</td>
<td>593 (per 554)</td>
<td>619 (per 204)</td>
<td>0.002</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean Viral Load (copies ml⁻¹)</td>
<td>4011 (per 2859)</td>
<td>8613 (per 392)</td>
<td>0.25</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.63 (per 24.60)</td>
<td>23.55 (per 24.37)</td>
<td>0.84</td>
<td>0.001</td>
</tr>
<tr>
<td>Prevalence of Opportunistic Infections (per cent)</td>
<td>186 (per 10) (86.51%)</td>
<td>168 (per 6) (71.79%)</td>
<td>0.00</td>
<td>0.0005</td>
</tr>
</tbody>
</table>
Figure 1. Kamps consort diagram 2016
Figure 2. Proportion of respondent’s adherence by various adherence methods at baseline

Figure 3. Proportion of adherent respondents by various adherence methods at 6 months
Figure 4. Comparison of CD4+ Cells at six months across intervention arms-kadoma mobile phone study (2016-17)

Figure 5. Comparison of respondents weights at baseline and six months By Intervention Arm Kadoma Mobile Phone Study (2016-17)

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Basic Principles and Applied GxP Regulations for ELISA Analytical Method Validation of Drugs and Biologics in FDA Driven Environment

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Abstract

The Food and Drug Administration (FDA) is a federal agency of the United States that is responsible for protecting the health of the public by ensuring the security, efficacy, and safety of both the veterinary and human drugs. The agency is also responsible for ensuring the safety of the food supply, radioactive materials, and cosmetics in the United States. The FDA has a statutory duty of ensuring that manufacturers of various products, including pharmaceuticals, comply with their respective established Good Manufacturer Practice (GMP).

ELISA (enzyme-linked Immunosorbent analysis) is an important bioanalytical assay format that pharmaceutical companies particularly use to validate in order to achieve a robust method suitable for the purpose of development of drugs and biologics in FDA regulated environment.

The analytical laboratories carrying out all toxicology or pharmacology as well as other pre-clinical studies for the purposes of making regulatory submissions are expected to comply with FDA’s Good Laboratory Practices (GLPs) and sound quality assurance principles throughout the validation of appropriate ELISA methods and/or testing process. As long as pharmaceutical companies evaluate analytical methods before and during regular use, they are not under any statutory obligation to comply with the FDA guidelines on method validation, including bioanalytical methods such as ELISA methods.

Keywords: FDA, GxP, Impurity ELISA, sandwich ELISA, validation. Basic Principles and Applied GxP Regulations for ELISA Analytical Method Validation of Drugs and Biologics in FDA Driven Environment.

Regulations

The Food and Drug Administration (FDA) is a federal agency of the of the United States Department of Health and Human Services (HHS) that is responsible for protecting the health of the public by ensuring the security, efficacy, and safety of both the veterinary and human drugs (Food and Drug Administration, 2017a). According to the Food and Drug Administration (2017a), the FDA is also responsible for ensuring the safety of the food supply, radioactive materials, and cosmetics in the United States. In brief, the FDA has a statutory duty of ensuring that manufacturers of various products, including pharmaceuticals, comply with their respective established Good Manufacturer Practice (GMP). In particular, the FDA has put in place the necessary mechanisms that are aimed at monitoring how drug manufacturers comply with its Current Good Manufacturing Practice (CGMP) regulations so as to guarantee the quality of drug products (Food and Drug Administration, 2017b). The Food and Drug Administration (2017c) explains that the CGMPs provide for systems that guarantee “proper design, monitoring, and control of manufacturing processes and facilities.” The significance of manufactures adhering to the CGMP regulations is that it assures the purity, quality, strength, and identity of drug products by requiring that medication manufacturers adequately control their manufacturing operations (Food and Drug Administration, 2017c). Specifically, this control of the manufacturing operations includes the establishment of strong quality management systems, acquisition of suitable quality raw materials, and an establishment of robust operating procedures, identifying and investing any deviations in product quality, and maintaining reliable testing laboratories (Food and Drug Administration, 2017c). The Food and Drug Administration (2017c) argues that if pharmaceutical companies adequately put into practice the CGMP regulations, it would help them to prevent incidences of deviations, mix-ups, errors, failures, and contamination. Consequently, this would guarantee that drug products from these pharmaceutical companies meet their quality standards.
Subsequently, in an effort to bolster the existing formal system of controls at pharmaceutical companies through the CGMP regulations, the Food and Drug Administration (2011) has established general principles and practices for the validation process. These general principles and practices, according to the Food and Drug Administration (2011), are suitable elements that pharmaceutical companies should use in process validation for the manufacture of animal and human biological and drug products, including the active pharmaceutical ingredients (APIs). In other words, the general principles and practices that the FDA has established the validation process incorporate the approaches and principles that all the manufacturers of medications can use to validate the manufacturing process. The Food and Drug Administration (2011) defines process validation as “the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product” (p. 4). The process entails a series of activities that take place over a product’s lifecycle and process. FDA guide on the general principles and processes for process validation describes the validation activities in three key stages: process design, process qualification, and continued process verification. It is important to point out that there has been a recent inquest as to whether the CGMPs require three successful process validation batches before a new API or a finished drug product is released for distribution (Food and Drug Administration, 2017d). In response, the FDA asserts that neither its policy nor the CGMP regulations specify a minimum number of batches to validate a manufacturing process (Food and Drug Administration, 2017d). Moreover, the existing FDA guidance on APIs also does not stipulate the number of batches for any process validation. Nonetheless, the FDA recommends that manufacturers should expand their testing based on the established validation protocol so as to provide an added guarantee that a batch satisfies all the appropriate and established criteria before API is used in the final drug product (Food and Drug Administration, 2017d).

Incidentally, the FDA has established a guideline that pharmaceutical companies should use to validate bioanalytical procedures such as high-pressure liquid chromatography (LC), gas chromatography (GC), combined LC and GC mass spectrometric (MS) procedures such as LC-MS-MS, GC-MS-MS, and LC-MS carried out for the quantitative determination of metabolites and/or drugs in biological matrices such as urine, serum, or blood (Food and Drug Administration, 2001). Food and Drug Administration (2001) adds that this bioanalytical method validation guidance for the pharmaceutical industry also applies to other bioanalytical procedures such as microbiological and immunological procedures, and to other biological matrices, for example, skin and tissue samples. Moreover, the guidance could be used in enzyme-linked immunosorbent assay (ELISA) tests.

**ELISA**

In order to fully understand the methodological approaches that the proposed research intends to use in evaluating the validation method for each of the two types of ELISA, it is imperative to first provide a brief background on sandwich (indirect method) ELISA and impurity ELISA. The sandwich ELISA, according to Adams and Moss (2007) and Ferenčík (2012), is a robust and sensitive method that measures the concentration of an antigen in an unknown sample. Ferenčík (2012) elucidates that the antigen of interest in sandwich ELISA is usually quantified between two layers of antibodies: the detection and capture antibody. The author adds that the detection and capture antibody must bind to the antigen’s non-overlapping epitopes. It is important to note that either the affinity-purified polyclonal or monoclonal antibodies can be utilized as the capture and detection antibodies (Adams & Moss, 2007). However, this is dependent on the cost, the dynamic range, and sensitivity of the final assay. Yamazaki et al. (2010) observe that sandwich procedures can sometimes be difficult to optimize and consequently, it is recommended that a tested and matching pair of antibodies should be used. The significance of using a tested matching pair of antibodies is that it guarantees that the antibodies are detecting various epitopes on the target protein in order not to interfere with another antibody binding.

The impurity ELISA format, on the other hand, quantify the residual impurities present in an assay. The residual impurities usually include Insulin, CHO HCP, rProtein A, and beta Glucan. In particular, the impurity ELISA methods used commercially available kits (particularly for early phase molecules) such as the spiking sample in matrix to evaluate the level of endogenous impurities in a sample. Different kits have different set-up and requirements for reagent handling.
Current industry practices

The bioanalytical methods in support of pharmacokinetic studies are considered to be among the most challenging types of analytical methods to develop and validate. Essentially, this means that ELISAs make up the more challenging bioanalytical methods to develop and validate. It is important to note that the selectivity and sensitivity of bioanalytical methods are fundamental to the success of both the pre-clinical and clinical pharmacology studies (Swartz & Krull, 2003). Swartz and Krull (2003) contend that just like any other analytical method, it is a requirement for the performance characteristics of a particular bioanalytical method to be shown by recorded laboratory data, to be reliable and reproducible to its intended purpose of use.

Subsequently, the Food and Drug Administration (2001) acknowledges that in a regulated laboratory such as that of a pharmaceutical company, the process of validating any type of analytical method does not start with the method in question. The software and any instrument that is used first in the validation process must be validated or qualified based on the current SOPs, and any data generated has to be maintained in compliance with the established FDA electronic and signature rules (Food and Drug Administration, 2001; Swartz & Krull, 2003). Swartz and Krull (2003) assert that it is only after the stage has been set that the validating process of a bioanalytical method can be separated into three parts: preparation of the reference standard, development of the bioanalytical method and the establishment of the procedure for the validated assay, and the application of the validated method in the regular drug analyses. After performing a full validation study on a bioanalytical method, laboratories often conduct partial validation to modify the existing validated analytical methods and cross-validation to compare two or more methods or to transfer methods. Nonetheless, the bottom line of the full and partial validation procedures is that using laboratory data to record to a particular method is appropriate and reliable for its intended use never changes. Fundamentally, this makes up the basic principle of method validation, regardless of the type of analytical method that is under scrutiny (Swartz & Krull, 2003, Shah, 2007; Galvao et al., 2008; Mattocks et al., 2010).

GAPS Identified

The FDA regulations such as the GLP, GMP, and other quality standards require that analytical methods should be evaluated before and during regular use. Moreover, there are no particular regulations on method validation. Nevertheless, the FDA, other government agencies, and industry task forces have developed guidelines for validating methods. Therefore, this means that as long as pharmaceutical companies evaluate analytical methods before and during regular use, they are not under any statutory obligation to comply with the FDA guidelines on method validation, including ELISA. It is incumbent upon these companies to either consider using the recommended FDA guidelines on method validation or to develop and implement a method validation that guarantees the security, efficacy, and safety of the drug product as provided under the FDA guidelines. Hence, pharmaceutical companies can, in fact, develop and adopt an ELISA test validation method that best suits their drug product

The FDA has only developed guidelines that can help pharmaceutical companies in developing and implementing a validation method for an analytic process such as the ELISA tests. Consequently, various pharmaceutical companies have developed different method validation processes for their analytical methods. Since the majority of the pharmaceutical companies have differences in handling the entire validation process, the emphasis should be on some basic concepts of validation in regular indirect ELISA assays as well as impurity methods for ELISA. For this purpose, a validation report should be represented, and a draft method has to be in place. All Validation activities must be in accordance and compliant with the International Conference on Harmonization (ICH) Q2 (R1) Guidelines for the Validation of Analytical.

Remedy and conclusion

The researchers shall place significant emphasis on examining the method validation steps of two types of ELISA: the binding ELISA format (sandwich ELISA) and impurity ELISA format.

Also, the FDA shall publish a detailed guidance on how to handle a validation/co-validation activities for both formats and/or establish clear criteria for a robust method validation process.
In order to ensure that the findings of the proposed research are reliable, the researcher shall conduct both the internal and external validity of the proposed study design. Fundamentally, the study’s internal validity shall entail the researcher addressing all the relevant parameters, and appropriate ELISA principles are implemented during the execution of the experiments. For example, the researcher shall ensure that the set parameters such as the incubation time, reagent dilution, coating concentration, and the standard curve parameters are performed as laid out in the established practices. In a nutshell, the researcher shall take all the necessary steps to protect the integrity of the study design. Incidentally, the external validity of the study design shall encompass co-validation of the proposed design with those that have been used in other laboratories. In other words, the researcher shall compare the findings drawn from the proposed research with those generated by similar studies from other laboratories. It is important to point out that the proposed research shall only compare its findings to those that have been produced using the ELISA test and not any other bioanalytical method. The coefficient variation (CV) that shall be used during co-validation shall be set below 20% for both laboratories and thereby conform to the traditional CV in most studies.

Furthermore, data from the experiment shall be subjected to statistical analysis so that the findings can be interpreted and appropriate inferences made from the research (Nachar, 2008; De Winter & Dodou, 2010; MacFarland & Yates, 2016). In particular, the researcher shall employ the Mann-Whitney U test to statistically analyze the collected data. The Mann-Whitney U test is described as the non-parametric alternative test to the traditional independent sample t-test (Nachar, 2008; De Winter & Dodou, 2010), Fay & Proschan, (2010). As a non-parametric test, the Mann-Whitney U test is used to compare two sample means that are drawn from the same population, and is used to determine whether two sample means are equal or otherwise (Black, 2009; Milenović, 2011). It is critical to point that in practice, the Mann-Whitney U test is used in statistical analysis when the assumptions of the t-test are not satisfied or the data in question is ordinal. Since the proposed research shall involve the use of samples with unknown antibody concentrations and some with impurities, it is easy to predict that the data is highly likely to be ordinal. Therefore, the Mann-Whitney U test would be ideal in the proposed research.

Moreover, a factorial design with center points for Binding ELISA format to set up appropriate parameters shall be used. In particular, the factorial design shall involve the selection of factors for the experiment, for example, the capture antibody concentration, detection antibody concentration, plate wash, reagent incubation time, specific dilutions of antibody, substrate incubation time. The importance of setting the parameters for the study is because these parameters shall be evaluated later during analytical method validation. The particular analytical method validation parameters that the proposed research shall seek to evaluate in so far as the sandwich ELISA is concerned to include specificity, accuracy, linearity, repeatability, Intermediate precision, Limit of Detection and QL (quantitation limit) and statistical analysis using JMP software analysis.

Depending on the needs of the user, the user must identify and select the method of ELISA analysis. The choice of the method analysis must be one that solves the analytical problem and shows its suitability for the intended purpose. Fundamentally, the aim of this proposed research is to evaluate the validation processes in in ELISA methods of analysis. An important part of the validation processes is to study and estimate all the parameters that are needed for good performance.

References


Evaluation of Effect of Labour Strikes on Patient Satisfaction in Secondary Health Institutions in Cross River State, Nigeria

Article by Samson Olusegun Aturaka¹, Ademola Amosu², Felix Sanni³, Musa Orenyi⁴, Margaret Dakwat⁵, Abiodun Olaiya Paul⁶, Opeyemi Joseph⁷

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³ Department of Chemistry, Federal University of Agriculture, Nigeria
⁴ Department of Supply Chain Management and Operations, Nigeria
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Abstract

The objective of the study is to evaluate the effect of labour strikes patient satisfaction in secondary Health Institutions in Cross River State, Nigeria. The study is cross-sectional descriptive study of 508 respondents form outpatient department, laboratory department, pharmacy department, Ante-Natal and Post-Natal clinic and ART clinic at the 7 secondary health institutions in CRS spread across 3 senatorial districts in the state between January and February 2018 using semi structured; self-administered, closed- and open-ended questionnaires that were divided into different sections each. Raw data were entered EpiData™ and exported for analysis using the SPSS software version 20. The data were cleaned and validated for use. Frequency tables were produced and associations between categorical variables were determined using chi squared test at a significance level of P<0.05.

Meanwhile, the negative effects of strikes are generally highly felt among all patients with no statistical significant difference whether employed, unemployed or retired (P>0.05). One of the effects of health workers’ strike is that strikes increase death rates and the result showed that regardless of patient’s education level, patients are fully aware that one of the effects of frequent strikes is increase in death rate (P>0.05). The result indicates that there is statistical significant difference in respondents’ responses between those without formal education, non-graduates and graduates. i.e. the higher the level of education, the higher the awareness that labour strikes affect the duty of health workers and have effect on patient’s attendance, poor healthcare performance and cause patients’ dissatisfaction (P<0.05).

Keywords: Labour Strikes, Patient Satisfaction, Access to Service Delivery, Quality of Care and Secondary Health Institutions.

Introduction

Nigeria masses have suffered from frequent healthcare workers’ strikes from 2012 to date from witnessing several health worker’s strikes involving all health professionals. Frequent healthcare workers’ strikes result from the closure of public healthcare institutions preventing Nigerians’ access to quality health services (Oleribe, 2016).

There are so many reasons health professionals go on strike. The main underlying causes of industrial action Nigeria include career stagnation, perceived discriminatory policies and demoralization from working in systems with poor infrastructure, manpower shortages and poor personal remuneration (Ogunbanjo, 2009). However, in recent times, there has been a divided opinion on true underlying factors responsible for the causes of industrial action (Botero, 2014).

The patient’s/client’s satisfaction in health institution is mainly identified by healthcare service delivery and quality of care. In health institution, satisfaction is not referred to as a specific product or service; it is composed by a combination of various features. Mostly in health institution, in healthcare
service delivery especially have regular contact with the patients. Therefore, evaluation and assessment of patients’ satisfaction is the paramount objective of healthcare institution (Hill & Alexander 2006). Meanwhile, Patients’ satisfaction has been recognized as a crucial parameter for measuring the quality of healthcare services (Lee, 2015).

The formation process of patients’ satisfaction starts with his/her goal setting in terms of service delivery and quality of care. As a rule, this goal is to meet varying patients’ needs. After the problem appearance the client starts to check the services available and compares analogues to choose the best one (Kotler et al. 2010).

**Materials and methods**

Using the sample size calculation formula for population less than ten thousand, (Olawuyi, 1996) sample size of 508 was calculated. Multistage sampling methods were employed from state ministry of health totaling 12 General hospitals. In stage one, 2 out of the 3 senatorial districts were selected by simple random sampling employing simple ballot in the two selected geopolitical zones. In stage two, 7 out of 9 secondary health facilities were selected from the 2 senatorial districts (Southern and Northern senatorial districts) by simple random sampling. In Stage three, questionnaires were distributed systematically to patients attending the following service delivery points: outpatient department, laboratory department, pharmacy department and ART clinic based on their client load. The patients were informed about the study. Those who agreed to participate were enlisted into the study by signing the consent form. Data collected were cleaned and validated for use. Simple frequency tables were produced and associations between categorical variables were determined using Chi square test at a significance level of P<0.05. Age, Gender, educational qualification, occupation and attendance at the facility were re-coded for the Chi-square analysis. Recoding of variables saw all participants grouped into male and female, graduates and non-graduates, married and single (with widows classified as singles) and attendance at the facility grouped into <1 year, 1- 5 years, 6-10 years, 11 – 15 years and > 15 years.

**Result and discussion**

**Table 1 Demographics**

Table 1. Socio-demographic characteristics of study participants

<table>
<thead>
<tr>
<th>SN</th>
<th>Gender of respondents</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>265</td>
<td>52.2</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>243</td>
<td>47.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SN</th>
<th>Marital status</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Single</td>
<td>248</td>
<td>48.8</td>
</tr>
<tr>
<td>2</td>
<td>Married</td>
<td>260</td>
<td>51.2</td>
</tr>
<tr>
<td>Age group</td>
<td>18 – 24</td>
<td>25 – 34</td>
<td>35 – 44</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>92</td>
<td>198</td>
<td>125</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Religion</th>
<th>Christianity</th>
<th>Islam</th>
<th>Traditional</th>
</tr>
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<tr>
<td>1</td>
<td>446</td>
<td>49</td>
<td>13</td>
</tr>
<tr>
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<td></td>
</tr>
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<td>3</td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Level of education</th>
<th>No formal education</th>
<th>Non-graduate</th>
<th>Graduate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83</td>
<td>220</td>
<td>205</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Employment status</th>
<th>Unemployed</th>
<th>Employed</th>
<th>Self employed</th>
<th>Retired</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>142</td>
<td>153</td>
<td>199</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
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</tr>
</tbody>
</table>

More than half of the respondents were out patients in their respective healthcare institutions, 345 (67.9%) while 163 (32.1%) were new patients at the time of interview. Majority of the respondents have been using the health their respective health facilities for the period of 1 – 5 years (202; 39.8%), approximately 25% (131) have been attending for less than a year, 25.4% (129) for 6 – 10 years, 7.9% (40) for 11 – 15 years while just 1.2% (6) have been using the hospital for over 15 years (Figure 3).
Table 2. Hospital attendance history of respondents

<table>
<thead>
<tr>
<th>SN</th>
<th>Patient status</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>New patient</td>
<td>162</td>
<td>32.1</td>
</tr>
<tr>
<td>2</td>
<td>Returning patient</td>
<td>345</td>
<td>67.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of years in attendance</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;1yr</td>
<td>131</td>
<td>25.8</td>
</tr>
<tr>
<td>2</td>
<td>1 – 5yrs</td>
<td>202</td>
<td>39.8</td>
</tr>
<tr>
<td>3</td>
<td>6 – 10yrs</td>
<td>129</td>
<td>25.4</td>
</tr>
<tr>
<td>4</td>
<td>11 – 15yrs</td>
<td>40</td>
<td>7.9</td>
</tr>
<tr>
<td>5</td>
<td>&gt; 15yrs</td>
<td>6</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Figure 3. Patient’s attendance status

More than half of the respondents were returning patients in their respective healthcare institutions, 345 (67.9%) while 163 (32.1%) were new patients at the time of interview.
Figure 4. Is this your first time in this hospital?

87 (17.1%) were in the hospital for the first time during while 421 (82.9%) have been visited the hospital more than once before the day of interview.

Patients’ satisfaction with health service during strikes

This section is to determine the level of patients’ satisfaction with health services during labour strikes. The question to ask here is “Can patients’ satisfaction be achieved when there are labour strikes? Majority of the respondents (447; 88%) were not satisfied with health care service during strikes, 92.1% were unhappy. Over seventy five percent (75.6%) go to private hospital during strikes while approximately ninety percent (89.8%) were not happy coming to government hospitals during strikes. Majority of respondents stated that strike is unnecessary in the hospitals (76.8%), 48.8% believed that strikes are too frequent in health institutions and 71.5% were unsatisfied because strikes contribute to increase in hospital bills (Table 8).

Table 3. Patients’ satisfaction with health service during strikes

<table>
<thead>
<tr>
<th></th>
<th>Agree</th>
<th>Disagree</th>
<th>Not Sure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labour strike cause patient’s dissatisfaction</td>
<td>447 (88.0%)</td>
<td>14 (2.8%)</td>
<td>47 (9.3%)</td>
</tr>
<tr>
<td>Patients are not happy during strike</td>
<td>468 (92.1%)</td>
<td>13 (2.6%)</td>
<td>27 (5.3%)</td>
</tr>
<tr>
<td>Will you be happy to come to this health facility during strikes?</td>
<td>52 (10.2%)</td>
<td>456 (89.8%)</td>
<td>-</td>
</tr>
<tr>
<td>Do you go to private hospital during strikes?</td>
<td>384 (75.6%)</td>
<td>124 (24.4%)</td>
<td>-</td>
</tr>
<tr>
<td>Is the strike in our hospital too frequent?</td>
<td>248 (48.8%)</td>
<td>189 (37.2%)</td>
<td>71 (14.0%)</td>
</tr>
</tbody>
</table>
Is the strike in the hospital necessary?

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>70</td>
<td>13.8%</td>
</tr>
<tr>
<td>No</td>
<td>390</td>
<td>76.8%</td>
</tr>
<tr>
<td>Don't know</td>
<td>48</td>
<td>9.4%</td>
</tr>
</tbody>
</table>

Does strike contribute to increase in hospital bills?

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>363</td>
<td>71.5%</td>
</tr>
<tr>
<td>No</td>
<td>115</td>
<td>22.6%</td>
</tr>
<tr>
<td>Don't know</td>
<td>30</td>
<td>5.9%</td>
</tr>
</tbody>
</table>

Comparison of the levels of patient’s dissatisfaction across gender, education and employment status

The level of patient’s dissatisfaction with health care services during strike is generally high (above 70%) across respondent’s gender, education and employment status as shown in table 9. Approximately 90% of female and 87% of male patients are not satisfied with cares receive during strikes. Across the level of education, the level of dissatisfaction is significantly higher (92.7%) than 88.2% dissatisfaction level found for non-graduates and 75.9% recorded for those without formal education (P<0.05). The level of dissatisfaction of patients during strike does not depend on employment status as dissatisfaction is very high across all status. Approximately ninety three percent (92.9%) dissatisfaction level was discovered among retirees followed by 89.5% among the employed, 88.4% among self-employed and the least value of 85.2% among the unemployed (p>0.05) (Table 9).

Table 4. Comparison of the levels of patient’s dissatisfaction across gender, education and employment status

<table>
<thead>
<tr>
<th>Gender of respondents</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>231</td>
<td>87.2</td>
</tr>
<tr>
<td>Female</td>
<td>216</td>
<td>88.9</td>
</tr>
</tbody>
</table>

X² 0.594, P value 0.743

<table>
<thead>
<tr>
<th>Level of education of respondents</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No formal education</td>
<td>63</td>
<td>75.9</td>
</tr>
<tr>
<td>Non-graduate</td>
<td>194</td>
<td>88.2</td>
</tr>
<tr>
<td>Graduate</td>
<td>190</td>
<td>92.7</td>
</tr>
</tbody>
</table>

X² 35.617, P value 0.000

<table>
<thead>
<tr>
<th>Employment status of respondents</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unemployed</td>
<td>121</td>
<td>85.2</td>
</tr>
<tr>
<td>Employed</td>
<td>137</td>
<td>89.5</td>
</tr>
<tr>
<td>Self-employed</td>
<td>176</td>
<td>88.4</td>
</tr>
<tr>
<td>Retired</td>
<td>13</td>
<td>92.9</td>
</tr>
</tbody>
</table>

X² 3.489, P value 0.745

Discussion

In the last five years, the Nigerian health system has experienced more than eight different strikes involving doctors, nurses and allied healthcare workers (Olatunji S., 2013; Hassan J., 2016). In fact, there has been an increase in the number of healthcare worker strikes across the country which are national, within the region or state based (Ogunbanjo, 2009; Olatunji, 2015 and Ibe, 2015) However, the felt implications of strikes are also supported by other studies (The Daily Newspaper, 2005).

Majority of the respondents (88%) were not satisfied with health care service during strikes, 92.1% were unhappy. Over seventy five percent go to private hospital during strikes while approximately ninety percent were not happy coming to government hospitals during strikes. Majority of respondents stated that strike is unnecessary in the hospitals (76.8%), 48.8% believed that strikes are too frequent in health institutions and 71.5% were unsatisfied because strikes contribute to increase in hospital bills. Approximately 90% of female and 87% of male patients expressed dissatisfaction with cares receive
during strikes. Across the level of education, the level of dissatisfaction is significantly higher (92.7%) than 88.2% dissatisfaction level found for non-graduates and 75.9% recorded for those without formal education. Approximately ninety three percent dissatisfaction level was discovered among retirees followed by 89.5% among the employed, 88.4% among self-employed and the least value of 85.2% among the unemployed.

Patient satisfaction is an indicator for performance management, it is a very important tool in processing monitoring and improving quality of healthcare services. Assesses what patient think about the care and treatment they have received present one approach to improve the quality of care (Donabedian, 1988). The findings in this study supported the earlier studies in the sense that 86.9% of respondents agreed that labour strikes causes poor healthcare quality, increase cost; it leads to loss of lives, loss of time, and loss of public confidence, low staff morale and results in wastage of our limited resources while 88.0% of respondents also agreed that labour strikes affect the duty of health workers and have effect on patient’s attendance, poor performance of healthcare and invariably lead to patients dissatisfaction. This shows that effects/impacts of labour strikes are favourably skewed towards healthcare workers since government will still pay for the number of days the workers remain on strike. The participants thus agreed from the study that labour strikes have negative effect generally on patients and their family. Poor healthcare quality is costly; it leads to loss of lives, loss of time, and loss of public confidence, low staff morale and results in wastage of our limited resources (Offei, 2012).

**Recommendations**

To avoid frequent strikes in health facilities, the most frequent recommendation cited by respondents was that government should take steps to resolve crisis and negotiate with the health workers union on time (97.8%). Another major recommendation is that government should take steps to see that basic facilities are in place and ensure that emergency teams are working (96.7%). Other recommendations include “health workers should work together in harmony and learn to relate with one another peacefully” (96.1%), “regular training should be organized for leaders in health institutions” (93.9%), “it should not be compulsory for health workers to join labour unions” (62.8%) and “healthcare/hospital workers should be restricted/ban from going on strikes” (60.0%). In the study of Obinna and his team, it was reported that respondents believe that strengthening of the healthcare system (82.7 %), improving financial and professional motivation of health workers (50.7 %) and involving healthcare workers in decision-making (43.3 %) were workable solutions to the plethora of strike action in Nigeria (Obinna et al., 2016).

**References**


Validity of Modified Early Obstetric Warning System (Meows) In Low Resource Setting: A Case of St. Francis Hospital Nsambya, Kampala, Uganda

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Abstract

Introduction: Globally every day, about 830 women die due to complications of pregnancy and child birth. Of these deaths, 99% occur in low-resource settings, and most could be prevented. Use of Modified Early Obstetric Warning System (MEOWS) would be appropriate. MEOWS is a monitoring chart intended to identify mothers at risk and initiate the right action, at right time by the appropriately skilled clinicians, at a time when treatment might make a difference to reduce maternal mortality and morbidity.

Objectives: To determine the sensitivity, specificity and predictive values of Modified Early Obstetric Warning System (MEOWS) in correctly identifying women at risk of developing obstetric morbidity in St. Francis Hospital_Nsambya between January and February, 2016.

Methods: The study was a prospective cohort study conducted at St. Francis Hospital Nsambya, maternity ward, from January to February 2016. MEOWS monitoring tool was used alongside with questionnaires.

Result: 502 respondent mothers were enrolled in the study. 160 patients (31.9%) triggered and of which: 11.5% of them had obstetric morbidity which included postpartum haemorrhage-35.5%, pre-eclampsia-26.3%, suspected infection-22.4%, third degree perineum tear-5.3%, anaesthetic complications-4% and prolong hospital stay-7%. MEOWS was 81.7% sensitive (95% CI 80-94%), 76.3% specific (95% CI 74-81%), with a positive predictive value 36.3% (95% CI 31-44%) and negative predictive value of 96.2% (95% CI 94-99%).

Conclusion: MEOWS chart is even effective for use in low resource setting, like Uganda.


Introduction

Background

Globally, about 830 women die daily due to complications of pregnancy and child birth. 99% of these deaths occur in low-resource settings. The risk of a woman in a developing country dying from a maternal-related cause during her lifetime is about 33 times higher than that of a woman living in a developed country. Most of these deaths are preventable (Say et al., 2014). Melanie (2014) argued that the principles underpinning the use of MEOWS is that patients who develop serious illness will usually display abnormalities in simple physiological parameters. Further, that if these early signs are recognized and appropriate escalation and intervention is undertaken, patient outcomes will be improved.

Uganda is one of the countries that failed to achieve the millennium development goals target of reduction of maternal mortality. The Maternal Mortality Ratio (MMR) is still high in Uganda at 438 / 100,000 live births (Uganda Bureau of Statistics [UBOS] and ICF International, 2012). About 15% of pregnant women develop complications during their pregnancy irrespective of their geographical locations. A great difference in maternal death between developing and developed countries is due to failure of the health care system to respond when complications arise (WHO, 2005).
One target under the Sustainable Development Goal (SDG) three is to reduce the global maternal mortality ratio to less than 70 per 100,000 births. It’s argued that no country should have a maternal mortality rate of more than twice the global average (United Nations [UN], 2015).

The early detection of severe illness in pregnant women remains a challenge to many clinicians involved in their care. The relative rarity of such events, combined with the normal changes in physiology associated with pregnancy and childbirth, compounds the problem. Abnormal physiology is common among hospitalised patients. However, clinical and physiological deterioration is evident for 6-8 hours before critical illness. Serious morbidity and mortality occurs after a period of slow and progressive physiological deterioration that goes unrecognized or is inadequately treated (Marc, Helen and Lauren, 2013)

Despite pregnancy and labour being normal physiological events, monitoring of vital signs is an integral part of maternity care. Regular recording and interpretation of these observations aids in the recognition of any changes in a woman’s condition. There are several bedside score and track-and-trigger systems designed to aid nurses/midwives in observation of vital signs. This is aimed at facilitating early recognition of a patient’s deterioration (Cioffi, 2000a).

In the United Kingdom (UK), a modified early obstetric warning system (MEOWS) has been used in obstetric inpatients to track maternal physiological parameters, and to aid early recognition and treatment of clinical deterioration. This followed a recommendation by the 2003–2005 Confidential Enquiry into Maternal and Child Health report (Lewis, 2007).

MEOWS track the following parameters: Temperature, Blood pressure, Heart rate, Respiratory rate, Oxygen saturation, Conscious level and Pain scores. The values of the observations are then translated into a summary score which has a critical threshold, above which medical review and intervention is required. Using MEOWS prompts early medical evaluation and referral to the appropriate practitioner.

There also, exist, a number of challenges associated with implementing MEOWS. A study by Isaacs et al. (2014) found the following challenges: Staffing pressures preventing adequate completion of charts (35%), Lack of support for EWS charts from midwives (22%) and Lack of teaching/training (21%). Other challenges were concurrent use of a standard vital signs chart/partograph (20%), too time-consuming (14%), lack of support for MEOWS charts from doctors (9%), Lack of evidence and validation of MEOWS in obstetrics (9%), impact on the mother of frequent interruptions (8%) and Poor correlation of charts with obstetric physiology (7%)

Problem statement

Maternal morbidity and mortality are highest around the time of delivery and early postpartum period (WHO, 2005). This is, predominantly, due to postpartum haemorrhage, preeclampsia and sepsis, which are all preventable if detected early. Early detection calls for closer monitoring of both mother and baby during this time.

Monitoring of obstetric inpatient is a problem as cited by Centre for Maternal and Child Enquiries (CMACE) report during their enquiry into maternal death in the United Kingdom (Lewis, 2007). Similar report was release by Ministry of Health (MOH) _ Uganda (MOH, 2010).

Locally the tools used do not guide the clinicians well enough on abnormal measurements. But even then, it depends on the clinicians’ knowledge of an abnormal test result. A MEOWS is a tool that guides the clinician based on colour codes for an abnormal result.

MEOWS is a monitoring tool recommended by CMACE for predicting mothers at risk of developing maternal morbidity and is currently being used in UK hospitals. However, no such tool has been validated for use in low income setting and data on the use of such tools is limited.

The researchers thought of it as critical to determine the applicability of MEOWS as an essential tool in the prediction of maternal morbidity in the context of developing countries such as Uganda.

Conceptual frame work

Soon after delivery (both Spontaneous Vaginal Delivery (SVD) and Caesarean Section (C/S)), the mothers are monitored using Modified Early Obstetric Warning Score (MEOWS). The result is either a ‘Triggers’ or ‘No triggers’. In case of no triggers, the doctor monitors the mother after 6-hour. In case of triggers (1Red and 2Yellows), the doctor is called to review the mother immediately. At discharge,
the doctor reviews the chart for evidence for any maternal morbidity or mortality. Refer to figure 1 below:

![Figure 1. Flow of events during application of MEOWS](image)

**Objectives**

The study had both general and specific objectives as below;

**General objective**

The general objective was to validate the usage of MEOWS as a tool for predicting risk of developing maternal morbidity in Uganda

**Specific objective**

The specific objective was to determine the sensitivity, specificity and predictive values of MEOWS in correctly identifying a woman at risk of developing obstetric morbidity in St. Francis Hospital Nsambya. Between January and February, 2016.
DOI: 10.21522/TIJCR.2014.05.01.Art006
ISSN: 2520-3096

Justification of the study

Modified Early Obstetric Warning System (MEOWS) was introduced with the aim of improving patients monitoring. It was first used in UK hospitals as monitoring tool to correctly identify women at risk of developing obstetric morbidity. Early recognition and treatment could reduce maternal morbidity and death. However, no such tool has been validated for use in low resource setting and data on the use of such tools is limited. It is critical to determine the applicability of MEOWS as an essential tool in the prediction of Maternal Morbidity in the context of the developing country like Uganda. This information might be useful to both clinicians and policy makers in reducing maternal Morbidity and death.

Research methodology

Study design

This was prospective cohort study carried out between January and February 2016.

Study setting

The study was conducted in the obstetrics and gynaecology department of St. Francis Hospital Nsambya. The hospital is a Catholic founded Private-Not-For-Profit hospital located in the Southern part of Kampala city. It is about three kilometres from the city central business area. It is a tertiary referral hospital with a bed capacity of 361. Its designated catchment area is Makindye West Health Sub-District and it’s owned by Kampala Catholic Archdiocese.

The clinical staffs working in the hospital’s maternity wards include: Obstetricians / Gynaecologists, Senior House Officers on specialty training, Intern Doctors, Registered Midwives, Enrolled Midwives and Nursing Assistants

Study population

All postnatal mothers who had delivered from St. Francis Hospital Nsambya during the study period.

Sampling

Postnatal mothers who consented for the study were recruited using consecutive sampling method until the required numbers.

Sample size

Buderer’s formula were applied to calculate the sample size as follows (Buderer, 1996)

\[
\text{Sample size estimation (n)} = \left( \frac{Z^2 \times \text{Prevalence}}{\varepsilon^2 \times S_n (1-S_n)} \right) = \left( \frac{1.96^2 \times 0.89 \times (1-0.89)}{0.05^2 \times 0.3} \right)
\]

\[\Rightarrow n = 501.45685 \]

\[\Rightarrow n = 502 \]

Where;

\[S_n = \text{Anticipated sensitivity of 89\% = 0.89 (Singh, McGlennan, England and Simons, 2012)}\]

\[\text{Prevalence} = \text{Prevalence of disease in population, which can be obtained from previous literature = 30\% = 0.3 (Singh, McGlennan, England and Simons, 2012)}\]

\[\varepsilon = \text{Required absolute precision on either side of the sensitivity = 5\% = 0.05}\]

\[Z_{1-\alpha/2} = \text{standard normal confidence level of 95\% = 1.96}\]

Inclusion criteria

Postnatal mothers who delivered from St. Francis Hospital Nsambya and consented for the study were included.

Exclusion criteria

Postnatal mothers who did consent for study or mothers below 18 years were excluded from the study
Study tools and techniques

During the study, the following tools were used in data collection:

I. **MEOWS chart:** This was adopted from the seventh Confidential Enquiry into Maternal and Child Health (CEMACH) report 2003-2005 (Lewis, 2007).

II. **The study questionnaire:** This was a semi-structured questionnaire in which data from the MEOWS chart and clinical notes was entered.

Pregnant mothers were recruited in the study by the Research Assistant (RA) in the labour suite when they come for delivery and observation on MEOWS started immediately after delivery. Research assistants had the respondents briefed concerning their roles and expectations during the research.

Data was collected using an interviewer questionnaire with open and closed ended questions from between January and February 2016 by trained Research Assistant and Principal Investigators. Data was extracted from the clinical notes during the follow up by the Principal Investigators for any evidence of obstetrics morbidity. All data were then concentrated to the Principle Investigator who later verified entries made in the data collection tools to confirm correctness of entries into the corresponding fields and to enable processing of data.
Figure 2. Copy of MEOWS Chart
Ethical procedures and considerations

The following ethical procedures were undertaken:
I. Consultation with senior supervisors, other medical personnel and eventual testing of the MEOWS tool was done.
II. Training four midwives and two senior house officers as research assistants was done. They were trained on how to use the MEOWS chart and initiate a call out algorithm.
III. Pre-testing the MEOWS chart on postnatal mothers for one week until efficiency was 100% on procedural and responsibility arrangements by the research team was carried out. The Midwives and Doctors were made aware of the importance of accurate charting of the patients’ parameters, the need for mandatory callout and ensuring of the medical review by Doctors.

Study procedures and techniques

During the study period, the postnatal mothers were monitored immediately after delivery with MEOWS chart every two hours in the first 24 hours by midwives.

When postnatal mothers were triggered (1 red or 2 yellow), a Doctor was called urgently to review the patients on bedside and those who had no trigger were review at 6 hours post natal.

Measurement of temperature (axillary) was done with digital thermometer, blood pressure taken with mercury Blood Pressure Machine (Dekamet Accoson sphygmanomanometer made in England). Respiratory rate was taken by counting and timing with clock for one minute. Heart rate and oxygen saturation was taken with pulse-oxymeter. Consciousness level of mothers (AVPU: A: Alert of time, place & person, score white colour _ Normal, V: response to voice; score yellow _ moderate abnormal, P: response to pain & U: unresponsive both score red indicative of very abnormal) was taken.

Pain scores (0 = no pain at rest or on movement, 1 = no pain at rest, slight pain on movement, 2 = intermittent pain at rest, moderate pain on movement, 3 = severe pain at rest, severe pain on movement) was done. Pain score of 0-1 was coloured white and considered normal whereas score 2-3 was yellow and considered abnormal.

Thermometer, Blood pressure machine and pulse oximeter were validated on a daily basis for consistent measurement. Pulse oximeter was validated on daily by measuring four normal healthy adults for their oxygen saturation and pulse rate (considered valid when their Oxygen saturation is > 95-100% and pulse rate between 60-100 beat/minute (Ahrens, 2006)

Digital thermometer was validated on daily basis to measure the ice melting point of ice park got from the freeze, which was considered valid when it read 0.00-0.01 °C (Mangum et al., 1995).

Mercury sphygmomanometer was validated daily by checking technical features determining the accuracy of mercury sphygmomanometers. The following features were realized; the top of the mercury meniscus rested at exactly zero without pressure applied. To avoid Substantial errors, manometer was kept vertical during measurement and the air vent at the bottom of the manometer and was kept patent to prevent the mercury column from responding sluggishly and from overestimating pressure.

A trigger was defined as a single abnormal observation (red trigger), or the combination of two mildly abnormal observations (two yellows triggers). A trigger initiated urgent medical assessment by the Doctors and no trigger postnatal mothers were reviewed after 6hours for medical assessment and MEOWS score. Outcome at discharge (maternal morbidity, death, intensive care unit admission & discharged alive) was retrieved from hospital record and notes. The definitions of maternal morbidity were agreed jointly by the principal investigators and my Supervisors at the beginning of the study and the Doctors were the ones to confirm the morbidity.

Check list on MEOWS chart was done at 6 hours and 24 hours by principal investigator / research assistants for completeness. Appendix 3.0 shows the checklist.
Table 1. Table showing limit of triggers for MEOWS parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Red trigger</th>
<th>Yellow trigger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>&lt;35 or &gt; 38</td>
<td>35-36</td>
</tr>
<tr>
<td>Systolic BP; mmHg</td>
<td>&lt; 90 or &gt; 160</td>
<td>150-160</td>
</tr>
<tr>
<td>Diastolic BP; mmHg</td>
<td>&gt;100</td>
<td>90-100</td>
</tr>
<tr>
<td>Heart rate; b/m</td>
<td>&lt; 40 or &gt; 120</td>
<td>100-120</td>
</tr>
<tr>
<td>Respiratory rate; b/m</td>
<td>&lt; 10 or &gt; 30</td>
<td>21-30</td>
</tr>
<tr>
<td>Oxygen saturation %</td>
<td>&lt; 95%</td>
<td>-</td>
</tr>
<tr>
<td>Pain score</td>
<td>-</td>
<td>2-3</td>
</tr>
<tr>
<td>Neurological response</td>
<td>Unresponsive, pain</td>
<td>voice</td>
</tr>
</tbody>
</table>

Variables

The following were the primary outcome variables; postpartum haemorrhage, preeclampsia, suspected infection, pulmonary emboli, pulmonary oedema, ruptured uterus, emergency hysterectomy, anaesthetic complications, obstetric injury, prolong hospital stay, admission to intensive care unit and maternal death.

Data management, analysis and presentation

Questionnaires were cross checked at the end of each day to ensure correctness and completeness. Data was coded and double entered into EPI data version 3.1, cleaned and exported to SPSS version 16.0 for statistical analysis.

Sensitivity, specificity, positives predictive value and negative predictive value were calculated. The validity of MEOWS was calculated as follows as shown in table 2 below;

Table 2. Calculation of sensitivity, specificity and predictive values

<table>
<thead>
<tr>
<th>Trigger had morbidity</th>
<th>Trigger had no morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positive (TP)</td>
<td>False Positive (FP)</td>
</tr>
<tr>
<td>No trigger had morbidity</td>
<td>No trigger had no morbidity</td>
</tr>
<tr>
<td>False Negative (FN)</td>
<td>True Negative (TN)</td>
</tr>
</tbody>
</table>

1. Sensitivity = \[ \frac{TP}{(TP+FN)} \] x 100%
2. Specificity = \[ \frac{TN}{(TP+FP)} \] x 100%
3. Positive predictive = \[ \frac{TP}{(TP+FP)} \] x 100%
4. Negative predictive value = \[ \frac{TN}{(TN+FN)} \] x 100%

Quality control

This was enhanced by training of the 4 Midwives and 2 Senior House Officers as research Assistant and rehearsal of tasks. These were Midwives and Senior House Officers employed in St. Francis Hospital Nsambya who was not on duty. Weekly meetings between Research Assistants and Principal investigators were held to review experiences, performance or progress. The questionnaires were translated into Luganda and back into English: both were used as necessary. Spot checks were done, editing in the field and at the end of the day and reviewing the questionnaires for completeness were done.

Results

Introduction

A total of 517 postnatal mothers were recruited in the study and monitored with MEOWS chart by midwives every 2 hour in first 24 hours. Of these, 502 were analysed (97%). Of the 502 mothers, 160 triggered (1 red or 2 yellow) accounting for 31.9% and they had urgent medical evaluation by the doctor
whereas of 342 mothers didn’t, “no triggered” and they had medical evaluation done at 6 hours postnatal by doctors.

Both categories had their MEOWS chart reviewed and clinical note at discharged for evidence of obstetric morbidity. The results were as follows; Triggered had morbidity was 58 (TP), Triggered had no morbidity was 102 (FP), No triggered had morbidity was 13 (FN) and No triggered had no morbidity 329 (TN). See figure 2 below;

**Figure 2.** Flow chart of result summary

TP = True Positive, FP = False Positive, FN = False Negative and TN = True Negative

**Results on demographic characteristics**

Of the study participants, 59% of the respondents were of the age between 26 to 35 years with mean age of 28.2 years. Educationally; primary level was at 5.0% Secondary levels was at 52.1% and Tertiary levels was 41.7%. Tribe distribution of the postnatal respondents; 59.4% of the respondents were from the central region of Uganda and other regions accounted 37.0% whereas none Ugandans were 3.6%

The results on demographic characteristics were as shown in table 3 below;
Table 3. The demographic characteristic of participant

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of postnatal mothers monitored with MEOWS</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>159</td>
<td>31.6%</td>
</tr>
<tr>
<td>26-35</td>
<td>296</td>
<td>59%</td>
</tr>
<tr>
<td>&gt; 36</td>
<td>47</td>
<td>9.4%</td>
</tr>
<tr>
<td>Tribes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central region</td>
<td>299</td>
<td>59.4%</td>
</tr>
<tr>
<td>Other region</td>
<td>186</td>
<td>37%</td>
</tr>
<tr>
<td>None Ugandan</td>
<td>18</td>
<td>3.6%</td>
</tr>
<tr>
<td>Religion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protestant</td>
<td>114</td>
<td>22.7%</td>
</tr>
<tr>
<td>Catholic</td>
<td>232</td>
<td>46.1%</td>
</tr>
<tr>
<td>Muslim</td>
<td>62</td>
<td>12.3%</td>
</tr>
<tr>
<td>Born again</td>
<td>79</td>
<td>15.7%</td>
</tr>
<tr>
<td>SDA</td>
<td>9</td>
<td>1.8%</td>
</tr>
<tr>
<td>Level of Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5</td>
<td>1.0%</td>
</tr>
<tr>
<td>Primary</td>
<td>25</td>
<td>5.0%</td>
</tr>
<tr>
<td>Secondary</td>
<td>262</td>
<td>52.1%</td>
</tr>
<tr>
<td>Tertiary</td>
<td>210</td>
<td>41.7%</td>
</tr>
</tbody>
</table>

Results on obstetric history of respondents

Of the study participants; antenatal care attendant was at 99% (497), HIV positive were at 5.4% (27). Mode of delivery was as follows; 56.4% (283) had vaginal delivery, 43% (216) had caesarean section and 0.6% (3) instrumental delivery (vacuum- extraction).

The results on obstetric history of mothers were as follows;

Table 4. Obstetric history of respondents

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of postnatal mothers monitored with MEOWS</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antenatal care</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attended</td>
<td>497</td>
<td>99.0%</td>
</tr>
<tr>
<td>Not attended</td>
<td>5</td>
<td>1.0%</td>
</tr>
<tr>
<td>HIV status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>27</td>
<td>5.4%</td>
</tr>
<tr>
<td>Negative</td>
<td>466</td>
<td>92.8%</td>
</tr>
<tr>
<td>Unknown</td>
<td>9</td>
<td>1.8%</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>369</td>
<td>73.5%</td>
</tr>
<tr>
<td>4 and above</td>
<td>133</td>
<td>26.5%</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>283</td>
<td>56.5%</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>216</td>
<td>42.9%</td>
</tr>
<tr>
<td>Instrumental delivery</td>
<td>3</td>
<td>0.6%</td>
</tr>
</tbody>
</table>
Results based on morbidity factors of mothers

One hundred and sixty patients (31.9%) of the mothers triggered and of which; 11.5%(58patients) had obstetric morbidity, including postpartum haemorrhage 35.5%(21), preeclampsia 26.3% (15), suspected infection 22.4% (13), third degree perineum tear 5.3% (3), anaesthetic complications 4% (2) and prolong hospital stay 7% (4).

Results on sensitivity, specificity and predictive values

The Results on sensitivity, specificity and predictive values are as below;

Table 5. Calculation of sensitivity, specificity, positive predictive value and negative predictive values

<table>
<thead>
<tr>
<th>Trigger has morbidity</th>
<th>Trigger has no morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positive (TP) = 58</td>
<td>False Positive (FP) = 102</td>
</tr>
<tr>
<td>No trigger has morbidity</td>
<td>No trigger has no morbidity</td>
</tr>
<tr>
<td>False Negative (FN) = 13</td>
<td>True Negative (TN) = 330</td>
</tr>
</tbody>
</table>

Sensitivity = [ \( \frac{TP}{(TP+FN)} \) x 100% ] = [ \( \frac{58}{58+13} \) ] x 100% = 81.7 %

Specificity = [ \( \frac{TN}{(TN+FP)} \) ] x 100% = [ \( \frac{329}{329+102} \) ] x 100% = 76.3 %

Positive predictive value = [ \( \frac{TP}{(TP+FP)} \) ] x 100% = [ \( \frac{58}{58+102} \) ] x 100 = 36.3 %

Negative predictive value = [ \( \frac{TN}{(TN+FN)} \) ] x 100% = [ \( \frac{329}{329+13} \) ] x 100% = 96.2 %

Summary of findings

The results for this study versus the acceptable normal threshold ranges (in brackets) are as follow;

1. Sensitivity of MEOWS in predicting maternal morbidity was 81.7% (95% Confident Interval; 80%-95%)
2. Specificity 76.3% (95% Confident Interval; 74-82%)
3. Positive predictive value 36.3% (95% Confident Interval; 30-44%)
4. Negative predictive value 96.2% (95% Confident Interval; 94-99%).

Discussion, conclusion and recommendation

Discussion

The values of MEOWS in this study is consistent with other findings; Sensitivity 81.7% (95% Confident Interval; 80%-95%), Specificity 76.3% (95% Confident Interval; 74-82%), Positive predictive value 36.3% (95% Confident Interval; 30-44%) and Negative predictive value 96.2% (95% Confident Interval; 94-99%) as the results of Singh, McGlennan, England and Simons (2012).

In this study the specificity was 76.3% and is comparable to 79% done in UK by Singh, McGlennan, England and Simons (2012).

Conclusions

MEOWS chart can be used as a monitoring tool for predicting maternal morbidity, safe mother life and reduce the burden of severe morbidity and maternal mortality.

Recommendation

MEOWS chart should be adapted for use as monitoring tool for inpatients in maternity ward in low resource settings like Uganda.
References


“Method Validation Activities in GxP Regulated Environment”

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Abstract

The focus of this research is to evaluate the method validation processes in ELISA (enzyme-linked Immunosorbent assay), particularly in the development of drugs and biologics and subsequent method validations following strictly regulated rules in GxP controlled environment.

In an effort to bolster the existing formal system of controls at pharmaceutical companies through the CGMP regulations, the Food and Drug Administration (2011) has established general principles and practices for the validation process. These general principles and practices are suitable elements that pharmaceutical companies should use in process validation for the manufacture of animal and human biological and drug products, including the active pharmaceutical ingredients (APIs).

Keywords: ELISA, validation, GxP, FDA, critical reagents, regulations.

Introduction

The Food and Drug Administration (2011) defines process/method validation as “the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product”. The process entails a series of activities that take place over a product’s lifecycle and process. FDA guide on the general principles and procedures for process validation describes the validation activities in three key stages: process design, process qualification, and continued process verification.

Based on a summary of results outlined at the first and second Bioanalytical Method Validation (BMV) workshops that were held in collaboration with the American Association of Pharmaceutical Scientist (AAPS), the United States Food and Drug Administration (FDA), the International Pharmaceutical Federation (FIP), Health Protection Branch (HPB), and the Association of Analytical Chemist (AOAC), two main important outcomes were identified (Vinodh P, Shah, 2007). First set up the Acceptance Criteria (AC) for a Bioanalytical assay after a method has been developed and second - set up an in-study validation parameters such as accuracy, precision, selectivity, limit of quantification (LQ), and reproducibility, necessary for acceptability of the analytical method performance. Even though the first workshop had met very wide popularity among the pharmaceutical companies, it had never been published as an official document of the FDA. A draft guidance, based on the results from the first workshop was developed and published in 1999.

Another focus of this article is on the second workshop that was held in 2000, a year after the draft guidance was published. A second workshop, sponsored by AOAC and FDA was held in 2000 where all requirements for different types of Validation activities - a full Validation, partial Validation, and Cross Validation were briefly discussed and the result was a basis for FDA Guidance on Bioanalytical Methods Validation.

Thus, FDA has established a guideline that pharmaceutical companies should use to validate bioanalytical procedures such as high-pressure liquid chromatography (LC), gas chromatography (GC), combined LC and GC mass spectrometric (MS) procedures such as LC-MS-MS, GC-MS-MS, and LC-MS carried out for the quantitative determination of metabolites and/or drugs in biological matrices such as urine, serum, or blood (Food and Drug Administration, 2001). Food and Drug Administration (2001) adds that this bioanalytical method validation guidance for the pharmaceutical industry also applies to other bioanalytical procedures such as microbiological and immunological procedures, and to other biological matrices, for example, skin and tissue samples. Moreover, the guidance can be used in enzyme-linked immunosorbent assay (ELISA) tests as well. Since there is not unified pattern for performing validation of analytic methods such as an ELISA, and there is no clear instructions on how
to proceed with the validation activities on different ELISA platforms, a unified approach needs to be found and applied.

ELISA

Enzyme-linked immunosorbent assay (ELISA) is a serologic technique which is currently used as a diagnostic tool to detect variety of target molecules such as antigens, allergens and food contaminants. The main step in ELISA is the direct or indirect detection of antigen by antigen-specific antibody, called “capture antibody” that is immobilized directly on the surface of 96-well plate. Then, the antigen of interest is “sandwiched” between the capture and so called “detection” antibody or secondary, enzyme-coupled antibody. A chromogenic substrate, specific to the enzyme-associated antibody, yields a visible color change or fluorescence, indicating the presence of antigen. Since the fluorogenic substrates are with very high sensitivity, the levels of antigens can be very accurately measured by ELISA techniques. Quantitative or qualitative measures can be assessed based on such Hello colorimetric reading. There are several platforms of ELISA assays widely used not only in the medicine, as a diagnostic tool, but also in the industry mainly for research purposes.

Typically, ELISA is performed using 96-well plates which are able to passively bind different antibodies. Wash steps of the plates are always included in order to wash out the material that was nonspecifically bounded to the plate. After the first antibody was “coated” on the plate, a detection enzyme is be linked to the primary antibody or a secondary antibody can be introduced that specifically recognizes the primary ones. These detection enzymes are usually horse-radish peroxidase (HRP) or Alkaline Phosphatase (AP) (ThermoFisher Scientific, 2017). A corresponding substrate (TMB) is introduced in order the reaction to be visualized. The plate is read on a Plate Reader using appropriate validated and qualified software (SoftMax), OD values are measured and the concentration of the unknown analyte is determined, (ThermoFisher Scientific, 2017).

The most sensitive and robust format of ELISA is so called “sandwich” or “capture” ELISA format where the analyte (antigen), which will be measured, is quantified between both antibodies – capture and detection antibody. The analyte should have at least two antigen sites capable of binding to the corresponding antibodies. Thus it is considered as most commonly used format of ELISA because of it’s highly efficiency in antigen detection. Moreover, many commercially available kits with pre-coated capture antibody (monoclonal or polyclonal ones) are manufactured following the same principle (CHO HCP ELISA kit, Protein a ELISA kit, Insulin ELISA kit). The monoclonal antibodies usually distinguish a single epitope and this permits quantification of small differences in antigen (analyte) contrasted with polyclonal which has the ability to pull down as much of the antigen as possible. The advantages of “sandwich” ELISA format are many, including high specificity, flexibility and sensitivity (the sample does not need to be purified before analysis) as well as suitability for more complex samples.

Steps in performing either ID or Binding ELISA are similar and can be summarized as follow:
- Coating a 96-well polystyrene plate with capture antibody at a desired concentration, following by incubation step at either ambient temperature or 40C for established period of time.
- Blocking the remaining protein-binding sites that left unbounded by addition of blocking buffer (commercially available or prepared in-house) for certain period of time. The incubation time with the blocking buffer of a choice depends on the protocol used and the nature of the molecule.
- Addition of standards, quality controls, negative controls at concentrations, determined during the method development step. Spiked samples can be added in order to monitor the accuracy of the method performance.
- Incubation with detection and secondary antibody at determined concentration/dilution. The most commonly used enzymes for detection are horse-radish peroxidase (HRP) and/or Alkaline Phosphatase (AP) which are being visualized using the substrates 3,3’,5,5’-tetramethylbenzidine (TMB) and/or P-Nitrophenyl-phosphate (pNPP) respectively. The measurements (readings of optical density) are taken by a plate reader, which uses different wavelength of the spectrum. The wavelength used depends mainly on the substrate and its stability. For example, the readings can be taken at 450nm (using one wavelength) or it can be corrected by adding another wavelength such as 650nm.
The purpose of using the specific wavelength is to absorb the maximum from the samples/standards fluorochrome emission. However, this measure the non-specific emission from all the other materials in as well. Thus, the use of an irrelevant wavelength where the samples/standards will not give out signal is important to subtract signal that comes from these materials. Data analysis – using different platforms such as Excel, JMP, PLA.

Statistical evaluation of data is very important in order to show how accurate and reliable the method is. That is the reason why, a correct choice of Standard curve, appropriate assay parameters (diluent, blocking buffers, antibody), and sample/reagent concentration is very essential.

Concept of method development and method validation activities – steps and parameters evaluated

Preliminary DOE (design of experiment) should be set up in order to establish best parameters for the assay of desire. Method development is very long process which includes many steps and adjustments to the initially chosen parameters. In order the right parameters to be chosen, a Plackett-Burman factorial design could be used. It is the most commonly used design of 8 or 12 runs to evaluate between 5 to 11 factors. The design is applied usually in an early development stage where there is not sufficient knowledge about how the system work. The Plackett-Burman experimental factorial design is developed in 1946 by two statisticians – Robin Plackett and J. Burman and it is the main purpose is to find the active factors (variables) using as few experiments as possible. It is a design that screens out for the important factors (variables) that could potentially influence the output. Plackett-Burman factorial design should mainly be used when there is complete lack of knowledge about the factors (variables) and their interactions. Once the significant factors are available and a knowledge about the interactions is known, then multi factorial design is preferable, using SAS JMP software.

The main goal of each DOE strategy is to develop a robust potency assay with reduced number of experiments. Moreover, once the critical variables are found, DOE give us an advantage of making our assay robust enough. Some of the assay variables can be the coating concentration (concentration of the capture antibody); concentration of the detection antibody, pre-incubation and incubation time, blocking buffers used, and dilution buffers used. Once the variables are identified and experiments were completed, next step is optimization of DOE to find the optimal condition of the assay format. Last but not least, JMP analysis needs to be completed and overall prediction profiler should be applied. Profiling approach is needed in order to see what is going to happen if only one factor or many factors have changed. JMP provide a number of highly interactive cross-sectional views of any response, it gives an idea how the prediction model changes. The Profiler displays traces for each variable, it represents the predicted response as one variable is changed while the others are hold constant.

After the critical variables are set and the method is optimized based on data analysis, robustness experiments must be performed and data should be included. After all that steps are completed successfully, the validation of analytical method can be initiated.

Validation of analytical methods, including ELISA, is a confirmation and definite evidence that the exact requirements for the intended use are fulfilled. There are many publications, articles, books and guides which mainly focus on the topic of method validation, but there is no definite final protocol on how to perform this activity. One of the reasons is that the requirements for the different analytical methods are different on which are the essential parameters that need to be used. Most of the pharmaceutical companies have their own Standard Operating Procedures (SOPs) which describe step-by-step on how a validation of analytical method should be carried. Moreover, the same SOP (within the same laboratory, same pharmaceutical company) can provide altered instructions on how a validation should be held in different types of ELISA platforms such as ID, Binding and Impurity ELISAs.

Validation of analytical methods should follow certain criteria described in the Bioanalytical Method Validation (BMV) guidance. Most recently, an ICH M10, Concept paper final was published dated from 07Oct2016. This is a final endorsed concept paper draft which serve as a guide for Bioanalytical Method Validation which main idea is to resolve technical and scientific issues in BMV for method validation on chromatographic and ligand binding assays. This guidelines is still in development phase. The guideline provides recommendations on the regulatory requirements for bioanalytical methods and will provide a harmonization of current guidelines to support the drug development process. One of the issues pointed in this endorsed final concept paper is defining correctly each validation parameter
needed (specificity, reproducibility, sensitivity, precision, recovery, range, dilution linearity, stability), clarification on what type of validation will be used (full, partial or cross-validation), establish requirements for reference standard and quality controls, critical reagents as well as combine scientific experience and advancement of equipment/technology. Documentation needed for the purpose of the validation and reports describing the study sample analysis will be also other aspect of the endorsed concept paper. The main idea is harmonization of requirements for bioanalytical method development and its application in accordance with the requirements for bioanalysis in non-clinical and clinical drug development.

So far, the main parameters included in each validation activity are as follow: specificity, accuracy, linearity, including sample linearity, precision, repeatability, Limit of Detection (LD), Quantitation Limit (QL), range. Not all parameters has been examined when a validation for ELISA methods is needed. Some of the runs can be performed such a way, so the data can serve for the purpose of evaluation of other parameters. For example, the data gathered from the accuracy run can be used for the precision, range and linearity. The data from accuracy run can be used for establishing the range and QL.

Moreover, there are many differences on how the validation is lead when different ELISA platforms are available. Impurity ELISA methods often include the sample linearity runs where samples of interests are spiked into a matrix (know standard with known concentration) and then different dilution are prepared and analyzed. This way, the proper dilution factor for the sample of interest is established and same is used during the entire validation process. Furthermore, feasibility study is also important, often performed during the development stage.

**Specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. In order to ensure that the impurity/analyte is specifically determined by the specific test method and that the recovery is not affected by other compounds or sample matrix, spiked samples (samples of interest, spiked with certain amount of the impurity stock) and formulation buffers (buffer without the active ingredient) as well as assay diluent must be evaluated. Usually, the formulation buffer and the assay diluent are treated the same way as the sample, spiked at the same amount of the stock of interest (for impurities it could be either Insulin, rProtein A, CHO HCP or Glucan). If the amount of recovered impurity for all samples, formulation buffer and or diluent are same, the conclusion could be that there is no interference, meaning that the impurity is not affected by the sample matrix, assay diluent or ingredients in the buffer. For regular ELISA formats such as Binding or ID methods, spikes are not required, but another not-specific compound may be included to the run to ensure that there is no specific binding occurred. This would confirm the specificity of the method when only the analyte of interest specifically binds to the antibodies on the plate.

**Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. When using a combined experimental approach to obtain results for Linearity, Precision, Accuracy and Quantitation Limit, samples are usually spiked at different concentrations and analysis is repeated between couple of analysts. The recoveries of all spiked concentrations for each one of the samples is calculated and the %Coefficient of Variation (CV) of the mean value is calculated. Acceptance criteria for all experiment should be set up before the validation has started.

**Linearity**

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Usually two different types of linearity are assessed in many Impurity methods - sample linearity, where samples are spiked at different concentrations that falls within the method range, % Recovery is calculated and then plotted
against the nominal “target” concentration. Another approach is evaluation of assay linearity, where
recoveries from all used standards from the standard curve are taken and analyzed. For Binding and ID
ELISA formats, performing only assay linearity could be acceptable.

**Precision**

Under precision part there are major parameters - repeatability, intermediate precision and
reproducibility. The precision of an analytical procedure expresses the closeness of agreement (degree
of scatter) between a series of measurements obtained from multiple sampling of the same
homogeneous sample under the prescribed conditions. Repeatability expresses the precision under the
same operating conditions over a short interval of time. Repeatability is also termed intra-assay
precision/validity. Intermediate precision expresses within-laboratories variations: different days,
different analysts, different equipment, etc. and it is referred as an external precision/validity. Precision
may be expressed as the relative standard deviation (RSD) or the percent coefficient of variation (%CV).

Reproducibility is a measure of inter-laboratory variation; the use of the analytical method in
different laboratories (i.e., a collaborative study).

The evaluation of reproducibility is not required for qualification of an analytical method.

**Range**

The range of an analytical method is the interval between, and including, the upper and lower levels
of analyte that has been demonstrated to have a suitable level of precision, accuracy, and linearity.

QL – The quantitation limit of an individual analytical procedure is the lowest amount of analyte in
a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation
limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used
particularly for the determination of impurities and/or degradation products.

DL – The detection limit of an individual analytical procedure is the lowest amount of analyte in a
sample which can be detected but not necessarily quantitated as an exact value.

**Robustness**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small,
but deliberate variations in method parameters and provides an indication of its reliability during normal
usage. Usually the robustness part is completed during the method development phase, when all
essential parameters are already set. The concept of robustness study will be described later in this
thesis.

After all acceptance criteria is met, and a proper documentation (Validation Protocol that summarize
all experimental data) is on board, the validation is considered completed. In some cases, part of the
Precision/ Intermediate Precision could be given to another lab, receiving lab that will eventually own
the validated method and will carry all the responsible for any future executions, corrections, and
technical reviews. This is important step in a process called tech-transfer or transfer of already set,
validated and scientifically sound method which will be described later.

Moreover, all validation activities need to be in accordance with the International Conference on
Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH
harmonized tripartite guideline, and Validation of Analytical Procedures Text and Methodology Q2
(R1), November, 2005.

**Concept of robustness studies**

Robustness can be defined as the capacity to replicate the (analytical) method in different laboratories
or under different conditions without the existence of unanticipated changes in the obtained result(s),
and a robustness test as an investigational set-up to assess the robustness of a method. A synonym of
robustness is ruggedness.

The terminology robustness is now widely applied in the pharmaceutical world and is given by the
International Conference on Harmonization of Technical Requirements for the Registration of
Pharmaceuticals for Human Use (ICH).
Robustness experiments are executed with already established assay conditions. The concentrations of all antibodies used (capture, detection), the standard curve fit parameters, all dilution factors, diluents and blocking buffers as well as the incubation steps for each step of the method are well-known. Moreover, the system suitability criteria in terms of appropriate ranges of adjusted results, %Recovery, %StDev and %CV of each replicate analyzed should be established as well.

Even though the assessment of robustness study is not required by the ICH, the actual execution of the study would allow us to monitor the ability to reproduce the analytical method in different laboratories or under different conditions without any unexpected differences in results.

Different factors from the operating procedure are carefully chosen in order to observe the possible source of variability. Those factors are examined in a certain known range that slightly exceeds the variations that are expected from the analysts, performing the method in different laboratories (intra-variables). In such a way, those factors that disrupt the good method performance are being discovered and they must be strictly measured during the implementation of the method.

Concept of stability of drug product and critical reagents and their subsequent qualification

Critical reagents are crucial to the assay performance due to their unique characteristics. They can be binding reagents such as binding proteins, capture/detection antibodies, conjugated antibodies, and biotinylated probes, antibodies that are used as quality controls, such as positive and negative controls. Typically, reagents are produced via biological processes and most of them prone to lot to lot variability. This is the reason why a qualification/validation of them is needed in order to monitor long-term stability as well as quality.

Moreover, finding out different environmental factors such as exposure to light, temperature, low/high humidity, or chemical factors such as acidic, basic or oxidative media that could possibly influence the stability of the critical reagents and/or drugs is important, thus should be included in every process of validating a bioanalytical method. Stability study could possibly give a clear picture on how critical reagents and drug products can be held, what would be the desirable shelf life, and how quality changes under certain circumstances i.e. changing the pH of the media, exposure to UV light, hi/low pH etc. This is essential in order to provide information about re-test date and storage conditions. Short-term stability as well as long-term stability of critical reagents and drug products is essential and despite the lack of more detailed information about the stability study in the FDA and EMA Bioanalytical Method Validation Guidance for Industry, an indication about characterization and qualification shall be done for the intended purpose even though the degree of required characterization varies considerably.

Stability study should be execute as per ICH guidelines. ICH Q1B - photo stability studies for New Drug Substances and Products as well as following Q1A (R2) stability testing. The finalized stability guideline gives a direction on the basic testing protocol necessary to assess the light sensitivity and stability of new drugs and products as well as references on stability testing procedures including temperature, humidity and duration in climatic Zone I and II. The method validation qualifies all reagents used, but further usage of them needs more detailed analysis and characterization in terms of long-term stability testing. Accelerated stability study can be determine as well, but the choice depends on the product’s nature. All pharmaceutical companies have different approaches in qualifying critical reagents/ drug products. Moreover, corresponding SOPs (standard operating procedures) should be written and strictly followed. Qualification of critical reagents or drug products can be executed differently depending on the status of the evaluated compound. If a critical reagent/drug product has change in terms of lot number (batch) or formulation, a simple comparison between the original and the new lot or old formulation and the new formulation can be done. Acceptance criteria for the method of interest as well as the suitability criteria is compared and evaluated.

When a previous lot/formulation is not available and a new lot/formulation has to be introduced, more detailed qualification is needed and it strictly depends on the procedures outlined. A single-point stability study may be desirable.

A re-qualification of a critical reagent (same reagent over time, reagent in regular use) is performed in order to establish the new re-test date of that reagent following same method and passing all acceptance criteria for the assay. A ±3STD calculations are included in order to set a range of variability
as well as to monitor the performance of the method over time. Trend analysis must be presented and documented in order to monitor the performance over time.

**Concept of calibration/qualification of GxP instruments**

IQ/OQ/PQ should be performed prior initializing validation of methods. IQ stands for Installation Qualification, OQ for Operational Qualification and PQ is Performance Qualification. A basic requirement of good bioanalytical method validation is that analytical instruments used for the intended purpose must be appropriately installed, calibrated and maintained. IQ is a process of proving if the installation of the instrument is correct, ensuring that all components meet the approved specification. Moreover, all recommendations as per manufacturer should be fulfilled.

OQ is the process when a testing of the instrument is done in order to ensure that the system functions well, meets certain criteria as well as to check how the result of testing is recorded. PQ also called process qualification has the goal to ensure that the specified criteria can be achieved on a reliable basis over a long period of time. It is important in order to demonstrate the instrument performs according to specification, appropriate to its routine use. Usually the IQ/OQ/PQ of an instrument is performed by a vendor and regulated by the SOPs specific for each pharmaceutical company.

**Conclusions**

FDA had not established specific regulations on specific method validation. Instead, the FDA has only developed guidelines that can help pharmaceutical companies in developing and implementing a validation method for an analytic process such as the ELISA. Consequently, various pharmaceutical companies have developed different method validation processes for their analytical methods. Since the majority of the pharmaceutical companies have differences in handling entire validation process, the emphasis of this research proposal is to show some basic concepts of validation for ELISA. For this purpose, validation parameters should be evaluated for the purpose of a robust and scientifically sound method. All Validation activities must be in accordance and compliant with the International Conference on Harmonization (ICH) Q2 (R1) Guidelines for the Validation of Analytical.

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