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LABORATORY ANALYSIS REPORT

TRIVALENT ORAL POLIO VACCINE (tOPV)

for

THE MINISTRY OF HEALTH

Date of Sampling: 14th April 2015
Date of Report: 9th May 2015

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1.0 EXECUTIVE SUMMARY

The Ministry of Health through the Division of Vaccines and Immunization aims to increase access to immunization services nationwide, in order to reduce morbidity and mortality due to vaccine preventable diseases. This is in acknowledgement of the proven benefits of immunization in the prevention, control and even eradication of life threatening diseases over the years. Of particular importance is the reduction of infant and child morbidity and mortality in line with the United Nations Millennium Development Goals (MDG). The other major consideration is to implement the World Health Organization / United Nations Children's Fund (WHO/UNICEF) Global Immunization Vision & Strategy (GIVS) which challenges national governments to immunize more people, from infants to seniors, with a greater range of vaccines.

Kenya, is one of the countries that have committed to polio eradication by 2018. As a requirement to ensure that this polio eradication goal is achieved, the Global Polio Eradication Initiative (GPEI) requires all countries affected by polio to continue doing risk analysis on a quarterly basis and to conduct preventative campaign for any counties / districts that are considered high risk.

According to the Kenyan Ministry of Health, the preventative campaigns are important as they act as booster doses since some children do not lifelong immunity after the 3 routine doses. The campaigns are also important in ensuring that any newborns after the last campaigns and any missed children during routine immunization are vaccinated and immune to polio.

The Ministry of Health has therefore planned to conduct 2 preventative polio campaigns in 2015 based on the January 2015 risk analysis which identified a number of counties to be still at risk of polio importation. These campaigns have been planned to be conducted in April 2015 in 32 counties, and May 2015 in 11 counties. The campaigns will target 6,070,573 children in the April campaign and 1,878,470 children under 5 years in the May campaign.

Before the campaign is conducted and as part of its quality assurance program, The Ministry of Health would like to test for vaccines for the absence of beta human chorionic gonadotropin hormone (βhCG) and any other impurities that may be present in the vaccine.

1.1 OBJECTIVES OF THE LABORATORY ASSESSMENT

The Laboratory assessment had the following objectives:

1.1.1 Analyze the content of the vials submitted for the presence of βeta human chorionic gonadotropin hormone (βhCG)

1.4 EXECUTION OF THE ASSESSMENT

The assessment was carried out at as follows:

1.4.1 Sampling and Sample Submission

The polio vaccine samples for Laboratory analysis were submitted as follows:

- Lot 1
  Delivered to the Laboratory on 13th April 2015, 10 samples manufactured by Serum Institute of India

- Lot 2
  Delivered to the Laboratory on 22nd April 2015, 12 samples manufactured by Serum Institute of India

- Lot 3
  Delivered to the Laboratory on 29th April 2015, 7 samples manufactured by Haffkine B.P.C.L of Mumbai, India

- Lot 4
  Delivered to the Laboratory on 6th May 2015, 7 samples manufactured by Haffkine B.P.C.L of Mumbai, India

All samples received for the analysis were closed vials.

In total, 36 samples of the polio vaccine were analysed for quality assurance.

The samples comprised routine samples distributed among the medical facilities for routine polio vaccinations, and polio preventative campaign samples carried out according to the Ministry of Health risk analysis for counties identified to be at risk of polio importation. These campaigns are carried out jointly with the World Health organization (WHO) and the United Nations Childrens Fund (UNICEF).

The samples were as follows:

1.4.1.1 Routine samples - sampled on 22nd April, 29th April and 6th May manufactured by The Serum Institute of India and Haffkine B.P.C.L of Mumbai of India

1.4.1.2 Campaign samples - sampled on 13th April, manufactured by The Serum Institute of India.

1.1.2 Analyze the submitted samples for the following hormones:

1.1.2.1 Estrogen
1.1.2.2 Leutinizing hormone (LH)
1.1.2.3 Follicle stimulating hormone (FSH)

1.1.3 Full toxicological analysis of the vaccine, identifying any other impurities that may be present in the vaccines.

1.1.4 Production of a written report that includes the procedure used for analysis, relevant references applied to the analysis, citing publication(s)

1.1.5 To inform and interpret to the Ministry of Health, the findings from the laboratory analysis.

1.2 REFERENCES USED DURING THE LABORATORY ASSESSMENT

1.2.1 Purification of Human Chorionic Gonadotropin Hormone by Anion-Exchange High Performance Liquid Chromatography (HPLC)

1.2.2 Dissociation of human chorionic gonadotropin hormone (hCG) into its sub units - Pierce, Morgan et al 1971

1.2.3 World Health Organization (WHO), biological programs - oral polio vaccine

1.2.4 Analysis of Estrogens using a solid core HPLC column - Thermo Fischer Scientific, Runcorn, Cheshire, UK.

1.2.5 Journal of Liquid Chromatography, Volume 10, Issue 10, 1987 - High Performance Liquid Chromatography Analysis of Leutinizing Hormone, J. W Sutherland 1987

1.2.6 Journal of Liquid Chromatography, Volume 10, Issue 8, 2006 - Analysis of intact human follicle-stimulating hormone preparations by reversed-phase high-performance liquid chromatography, Loureiro RF et al.

1.3 MONOGRAPHS USED DURING THE ASSESSMENT

1.3.1 United States Pharmacopeia (USP)
1.3.2 British Pharmacopeia
1.3.3 Estrogen USP

1.3.2 **Sample Description**

The samples used for the Laboratory analysis were:

Date Submitted	Batch No.	Expiry Date	Manufacturer	Comments
Lot 1 13th April 2015	18104105	Sept. 2016	Serum Institute - India	Closed vial
	18104106	Sept. 2016	Serum Institute - India	Closed vial
	18104107	Sept. 2016	Serum Institute - India	Closed vial
	18104108	Sept. 2016	Serum Institute - India	Closed vial
	18104109	Sept. 2016	Serum Institute - India	Closed vial
Lot 2 22nd April 2015	18004086	Mar. 2016	Serum Institute - India	Closed vial
	18004088	April 2016	Serum Institute - India	Closed vial
	18004084	Mar. 2016	Serum Institute - India	Closed vial
	18004035	Jan. 2016	Serum Institute - India	Closed vial
	18004087	Mar. 2016	Serum Institute - India	Closed vial
Lot 3 29th April 2015	PV 1401004	Mar. 2016	Haffkine B. P. C. L - India	Closed vial
	PV 1404002	Mar. 2016	Haffkine B. P. C. L - India	Closed vial
	PV 1404003	Mar. 2016	Haffkine B. P. C. L - India	Closed vial
	PV 1404005	Mar. 2016	Haffkine B. P. C. L - India	Closed vial
Lot 4 6th May 2015	18004083	Mar. 2016	Serum Institute - India	Closed vial
	18004084	Mar. 2016	Serum Institute - India	Closed vial
	18004085	Mar. 2016	Serum Institute - India	Closed vial
	18004086	Mar. 2016	Serum Institute - India	Closed vial
	18004087	Mar. 2016	Serum Institute - India	Closed vial
	18004088	Mar. 2016	Serum Institute - India	Closed vial

1.4.2 Laboratory Analysis

Laboratory Analysis using High Performance Liquid Chromatography (HPLC) method for the determination of:

1.4.2.1 Human Chorionic Gonadotropin Hormone (β hCG) by Anion-Exchange chromatography

1.4.2.2 Estrogen and its compounds - estrone, estradiol, estriol and ethynylestradiol

1.4.2.3 Follicle stimulating hormone (FSH)

1.4.2.4 Leutinizing hormone (LH)

1.4.3 Reporting

Laboratory reporting and interpretation of the data was carried out on 8th May 2015.

2.0 SUMMARY OF FINDINGS

Polio is an infectious disease caused by a virus that lives in the throat and intestinal tract. It is most often spread through person-to-person contact with the stool of an infected person and may also be spread through oral/nasal secretions.

The trivalent oral polio vaccine (tOPV) contains an oral mixture of 3 viruses (Types 1, 2, and 3), selected by their ability to mimic the immune response following infection with wild polioviruses, but with a significantly reduced incidence of spreading to the central nervous system. Three or more spaced doses of OPV are required to generate adequate levels of seroconversion.

Human Chorionic Gonadotropin (hCG) hormone is synthesized by the chorionic tissue of the placenta and is found in urine during pregnancy. hCG is dimeric and is composed of two non-covalently bonded glycopeptides sub-units termed α (alpha hCG) and β (beta hCG).

A High Performance Liquid Chromatography (HPLC) method has been developed for the detection of hCG hormone sample in one chromatographic run using anion exchange chromatography. During the 60 minute linear gradient run, complete separation was accomplished in 40 minutes. The retention time for the hCG peak using this method was about 35 minutes.

The analysis of the hormones was carried out using the accupore phenyl X HPLC column, which can achieve the separation of structurally related aromatic steroids within a short retention time of about 5 mins.

Of the twenty nine (29) polio vaccine samples sampled and subjected to Laboratory HPLC analysis, the following findings were arrived at:

- Beta human chorionic gonadotropin (βhCG) hormone was not detected in any of the polio vaccine samples analysed
- Estrogen and estrogen compounds - estrone, estril, and ethynylestradiol were not detected in any of the polio vaccine samples analysed. **However estradiol was detected in 12 (twelve) vials submitted.**
- Follicle stimulating hormone (FSH) was not detected in any of the polio vaccine samples analysed
- Leutinizing hormone (LH) was not detected in any of the polio vaccine samples analysed.

$\left(\frac{12}{29} \times 100\right) = 41.4\%$

41.4 %

% all the tested vials had Estradiol

3.0 DESCRIPTION OF ANALYTICAL METHODS

3.1 HUMAN CHORIONIC GONADOTROPIN

3.1.1 Introduction

Human chorionic gonadotropin (hCG) hormone is synthesized by the chorionic tissue and is found in urine during pregnancy.

hCG together with luteinizing hormone (LH), follicle stimulating hormone (FSH, follotropin) and thyroid-stimulating hormone (TSH, thyrotropin) comprise the glycoprotein hormone family. All are dimeric and composed of two non-covalently bonded glycopeptide subunits termed α and β. The total number of amino acid residues in hCG for the α subunit is 92 and for the β 3 chain is 147. The amino acid sequences of both subunits of hCG have also been determined by Pierce and Morgan et al. Oligosaccharide and sialic acid chains are attached to both subunits. Each subunit is extensively crosslinked by intramolecular disulfide bonds.

A great number of immunological methods for hCG assays are available. These are based on haemagglutination, latex particle agglutination, complement fixation and radio immuno-reaction. The sensitivity and specificity of these methods make them potentially useful for the measurement of hCG hormone in urine concentration.

High separation of hCG has been obtained by simple methods of column chromatography on diethyl-aminoethyl (DEAE) Sephadex and Sephadex G-100 columns by Bell et al. Another method employing stepwise gradient with increasing NaCl concentration was reported by Yi-Han Chang et al. The isolation of HCG using these methods would be suitable but the procedures are time consuming, the standard column chromatographic procedures taking about two [2] weeks.

A rapid isolation of hCG can be accomplished in one day using anion exchange chromatography.

3.1.2 Description of the Analytical Method

The isoelectric point of the hCG molecule is about 4, solutions with higher pH contain HCG molecules in anionic form.

Eluent "A" was phosphate buffer [ph=6], while eluent "B" was 0.01M phosphate buffer [pH=3]. Buffer "B" contained 0.05M sodium sulfate and 0.05M sodium hydrogen sulfate. The pH of the solution was acidic due to the acidic character of the NaHSO4. Buffer "A" contained 0.01% v/v of "B" leading to the sulfate and hydrogen ion concentrations being 10,000 times

smaller. This means that during a 40 min linear gradient both the hydrogen ion and the sulfate concentrations have been significantly increased, conditions that promote the elution and good resolution of HCG.

The retention time of the HCG peak was about 35mins.

3.1.3 HPLC Apparatus and Columns

3.1.3.1 HPLC Equipment

The analytical HPLC system used in this assessment consisted of a Shimadzu Class VP 10 system. A Dell computer connected to Shimadzu SCL 10A - system controller was used for gradient control of the two shimadzu pumps.

The preparative procedures were accomplished on Shimadzu LC-10AT Liquid Chromatograph connected to a Shimadzu 10AV UV VIS detector with a model 201 fraction collector and equipped with a 7125 sample injection valve with 1ml sample loop.

3.1.3.2 Column

Anion exchange separations were performed on an ODS 5µm 250mm x 4.6mm column.

3.1.3.3 Materials

Mobile phases contained analytical - grade sodium acetate, sodium bicarbonate, potassium chloride, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium sulfate and sodium hydrogen sulphate.

HPLC grade acetonitrile and deionized water were used for preparing the eluents. Solvents were degassed ultrasonically.

3.1.3.4 Gradient Programs

The chromatographic run started isocratically by pumping 100% "A" mobile phase for 20min. A linear gradient from 0% "B" to 100% "B" mobile phase was employed at a 1.oml/min flow rate for 40 mins. The samples were dissolved in "A" eluent and injected at the beginning of the gradient run.

3.2 AROMATIC STERIODS

3.2.1 Introduction

Aromatic steroids can present a challenge in liquid chromatography as in reversed phase it is difficult to get good separation and the use of a highly selective phase is the key to overcoming this challenge.

To carry out this analysis, the Accucore Phenyl-X phase was employed to achieve the separation of four structurally related aromatic steroids classed as estrogens.

Estrogens are a group of steroids thus named for their importance in the estrous cycle. They function as the primary female sex hormone. Estrogens are used as part of some oral contraceptives and in estrogen-replacement therapy for postmenopausal women.

Three major naturally occurring estrogens in women are:

- estrone (E1),
- Estradiol (E2),
- Estriol (E3).

Estradiol (E2) is the predominant form in nonpregnant females, estrone is produced during menopause, and estriol is the primary estrogen of pregnancy.

Ethynyl-estradiol, a derivative of estradiol, is an orally bioactive estrogen used in almost all modern formulations of combined oral contraceptive pills.

While the standard C18 phase fails to separate these four compounds, the Accucore Phenyl-X HPLC column can baseline resolve them isocratically, providing good retention and unique selectivity

3.2.2 Description of the Analytical Method

Accucore HPLC columns use Core Enhanced Technology™ to facilitate fast and high efficiency separations. The 2.6 µm diameter particles are not totally porous, but rather have a solid core and a porous outer layer. The tightly controlled 2.6 µm diameter of Accucore particles results in much lower backpressures than typically seen with sub-2 µm materials.

The accucore Phenyl-X alkyl aromatic bonded phase provides a unique selectivity when compared to other reversed phase materials such as C18

or Phenyl. The advanced design of the bonded phase makes it robust and compatible with highly aqueous mobile phases.

The retention time for the aromatic steroids was less than 10mins.

3.2.3 HPLC Apparatus and Columns

3.2.3.1 HPLC Equipment

The analytical HPLC system used in this assessment consisted of a Shimadzu Class VP 10 system. A Dell computer connected to Shimadzu SCL 10A - system controller was used for gradient control of the two shimadzu pumps.

The preparative procedures were accomplished on Shimadzu LC-10AT Liquid Chromatograph connected to a Shimadzu 10AV UV VIS detector with a model 201 fraction collector and equipped with a 7125 sample injection valve with 1ml sample loop.

3.2.3.2 Column

Accupore Phenyl - X 2.6um, 100 x 2.1mm column

3.2.3.3 Materials

Mobile phases contained analytical grade - 15:40:45 acetonitrile:methanol:water.

Wash solvent was same as the mobile phase.

HPLC grade acetonitrile and deionized water were used for preparing the eluents. Solvents were degassed ultrasonically.

3.2.3.4 Gradient Programs

The chromatographic run started isocratically by pumping 100% "A" mobile phase for 20min. A linear gradient from 0% "B" to 100% "B" mobile phase was employed at a 1.oml/min flow rate for 40 mins. The samples were dissolved in "A" eluent and injected at the beginning of the gradient run.

4.0 RESULTS

4.1 Chromatograms for the determination of β hcg

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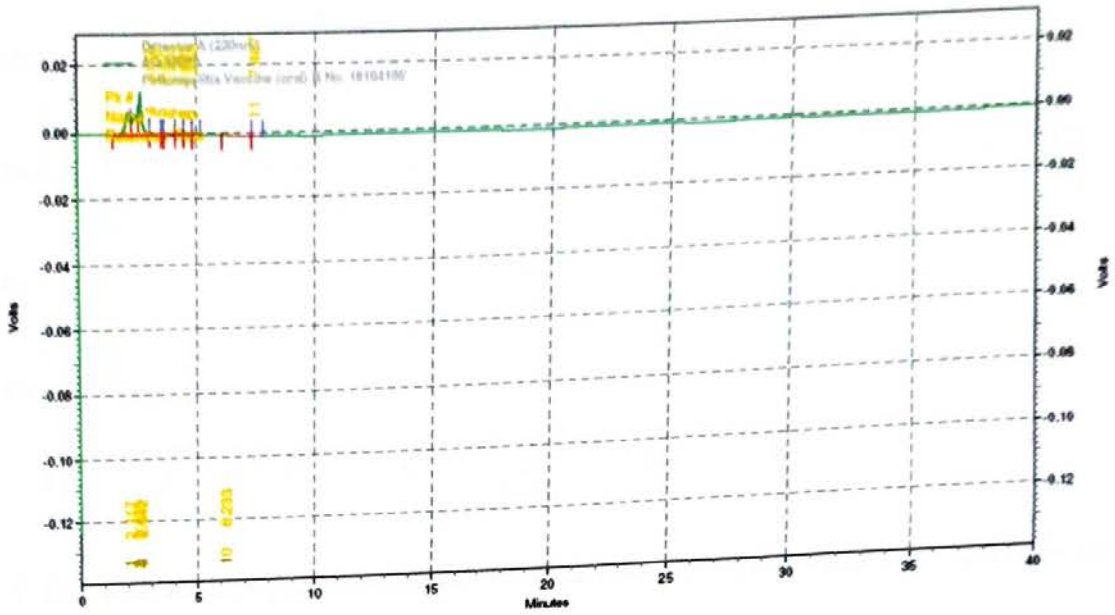

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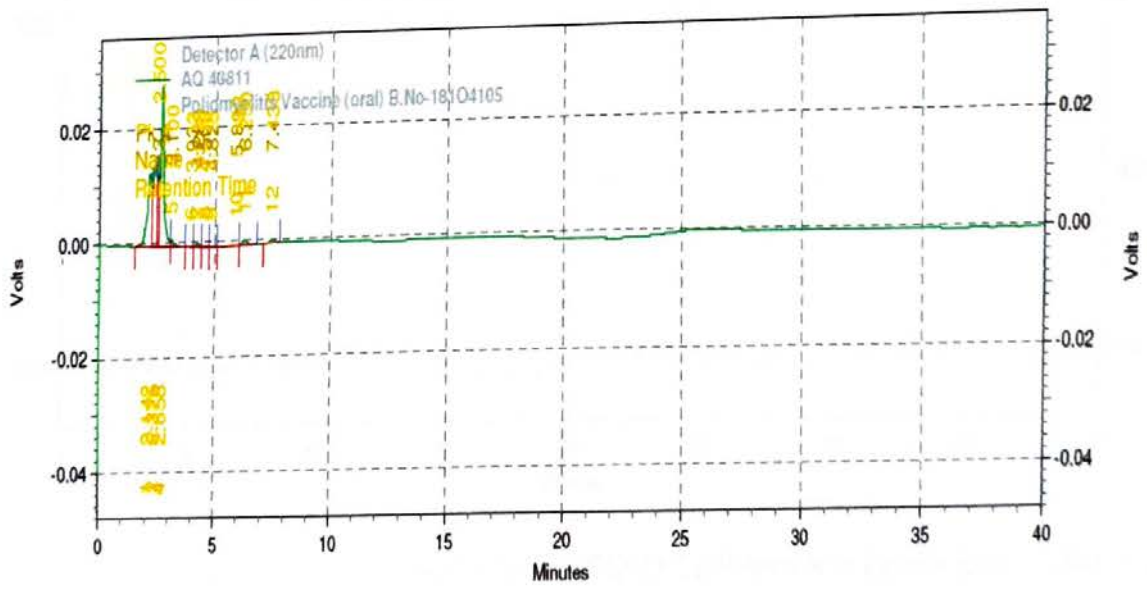
AQ 43811



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4.1 Chromatograms for the determination of β hcg

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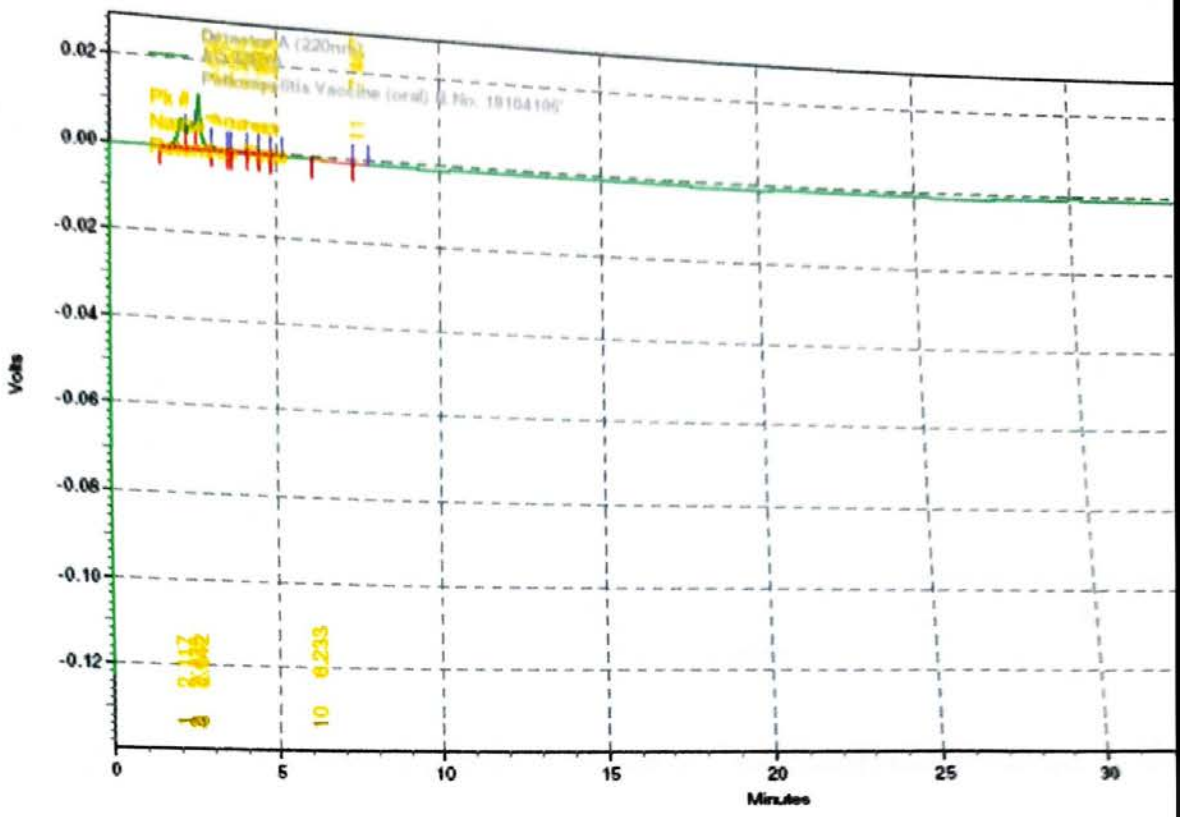

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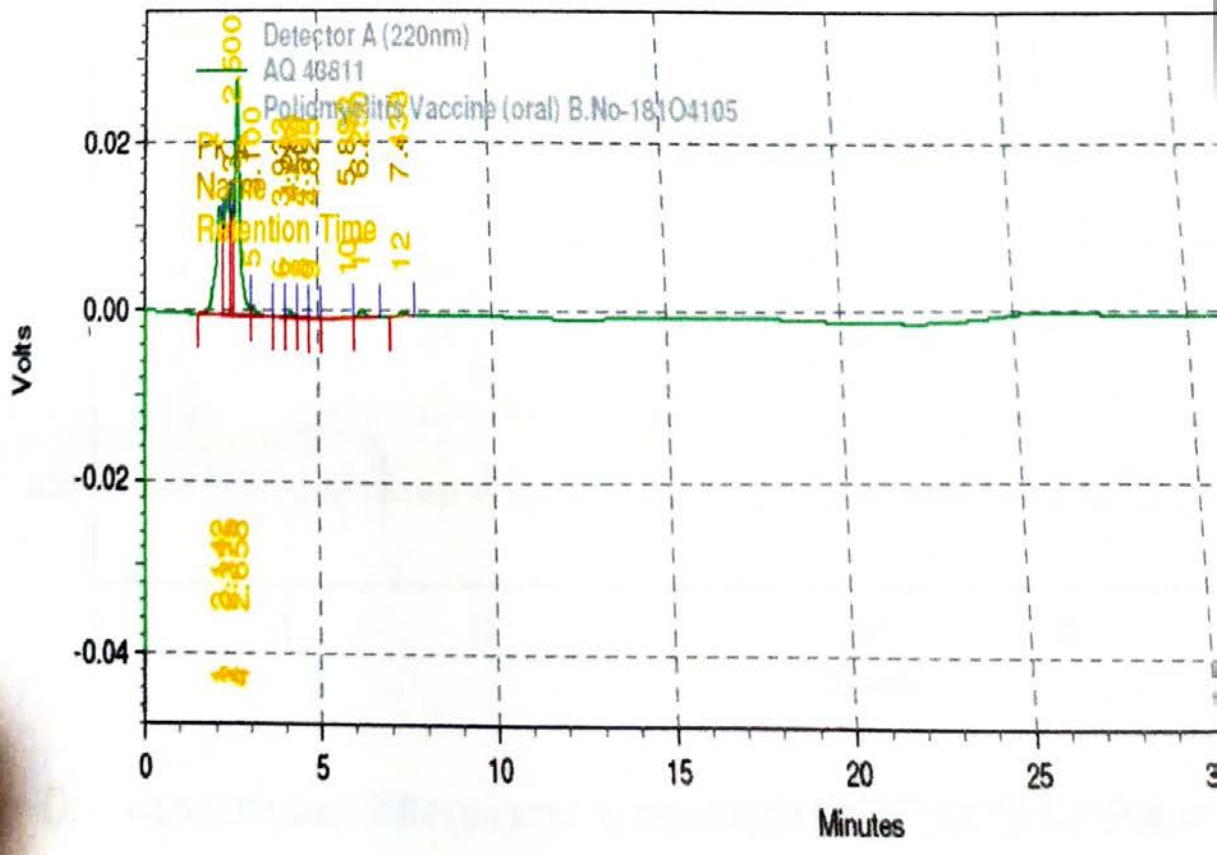

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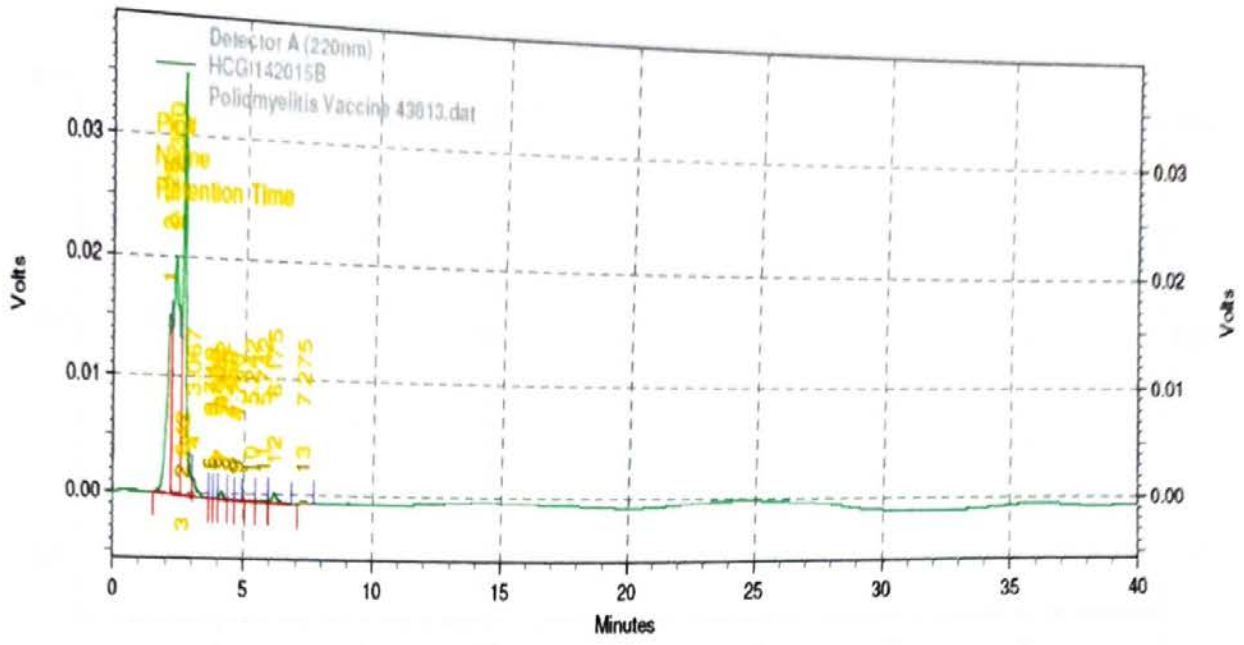

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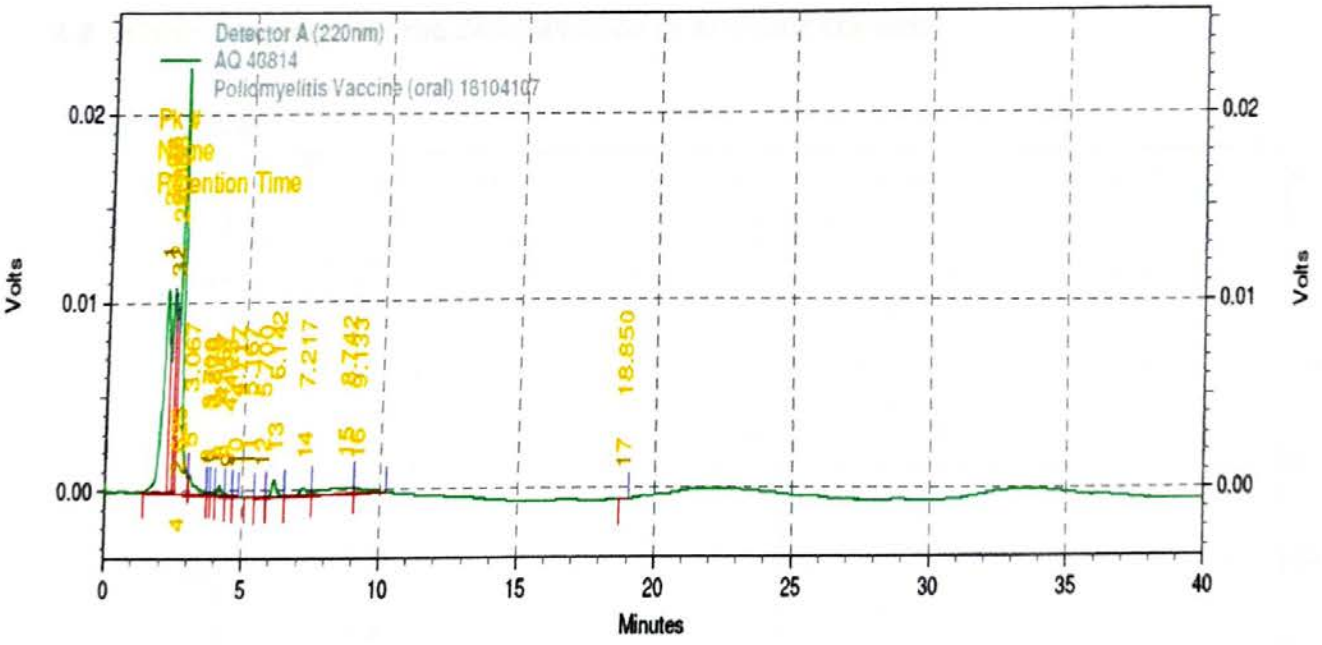

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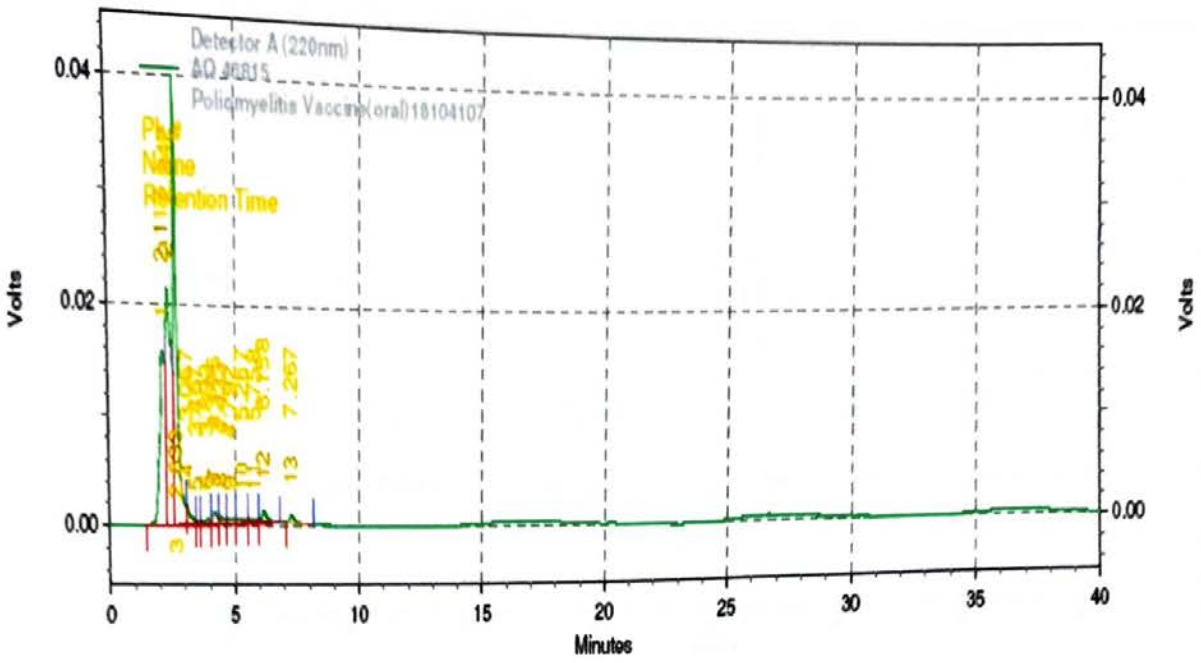
AQ 43815



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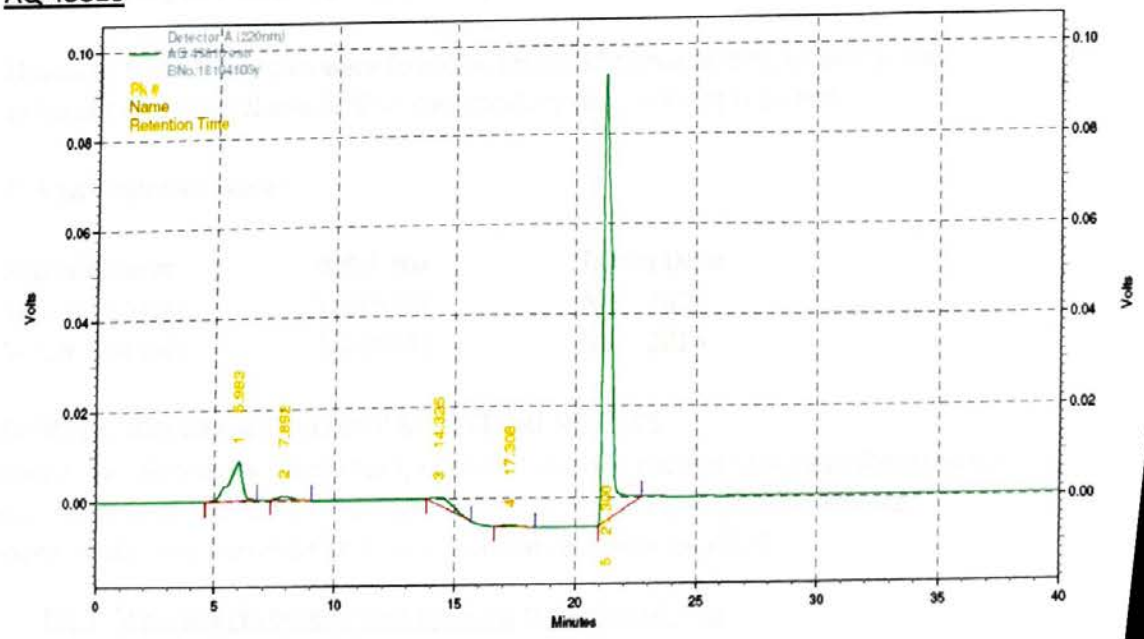
4.2 Chromatograms for the determination of Aromatic Steroids

AQ 43810



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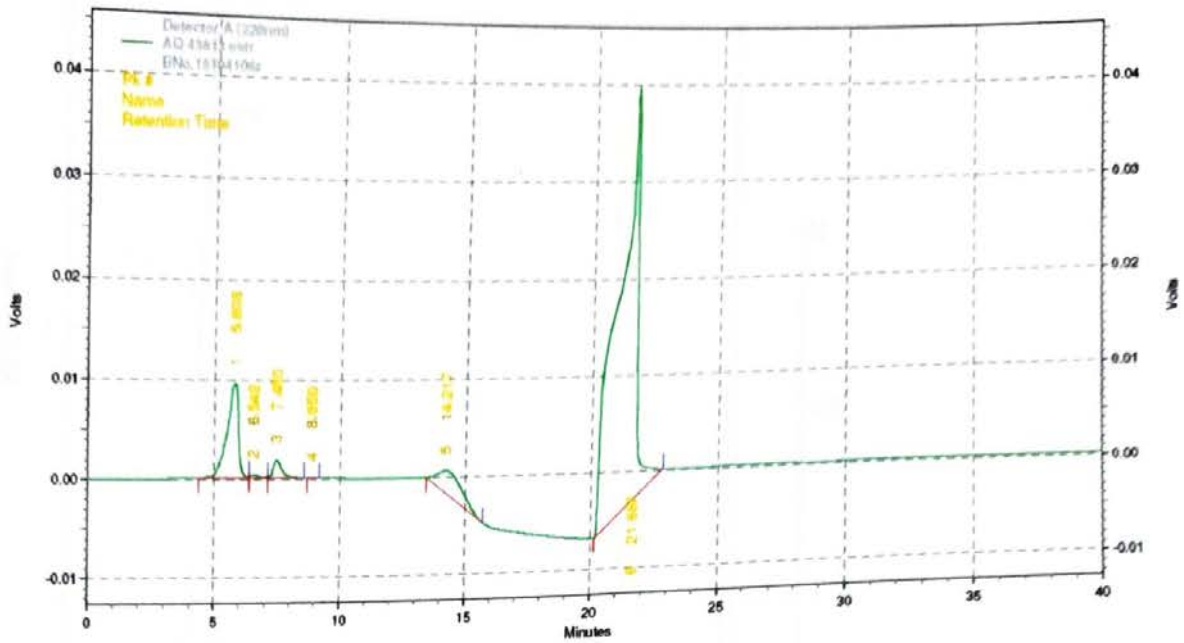

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4.3 Summary of Laboratory Results

Beta human chorionic gonadotropin hormone (βhCG) and glycoprotein hormones - estrogen, follicle stimulating hormone (FSH) and leutinizing hormone (LH) were not found in the polio vaccine samples analysed.

However two (2) samples were found to exhibit chromatograms similar to the presence of estradiol and further confirmatory tests may be required.

The two samples were:

Manufacturer	Batch No	Expiry Date
Serum Institute	18004086	Mar. 2016
Serum Institute	18004083	Mar. 2016

5.0 INTERPRETATION OF LABORATORY ANALYTICAL RESULTS

Following the Laboratory assessment, none of the polio vaccine samples submitted for analysis were found to contain human chorionic gonadotropin (hCG) hormone, estrogen, leutinizing hormone or follicle stimulating hormone (FSH).

Fig 1. Standard chromatogram showing presence of hCG

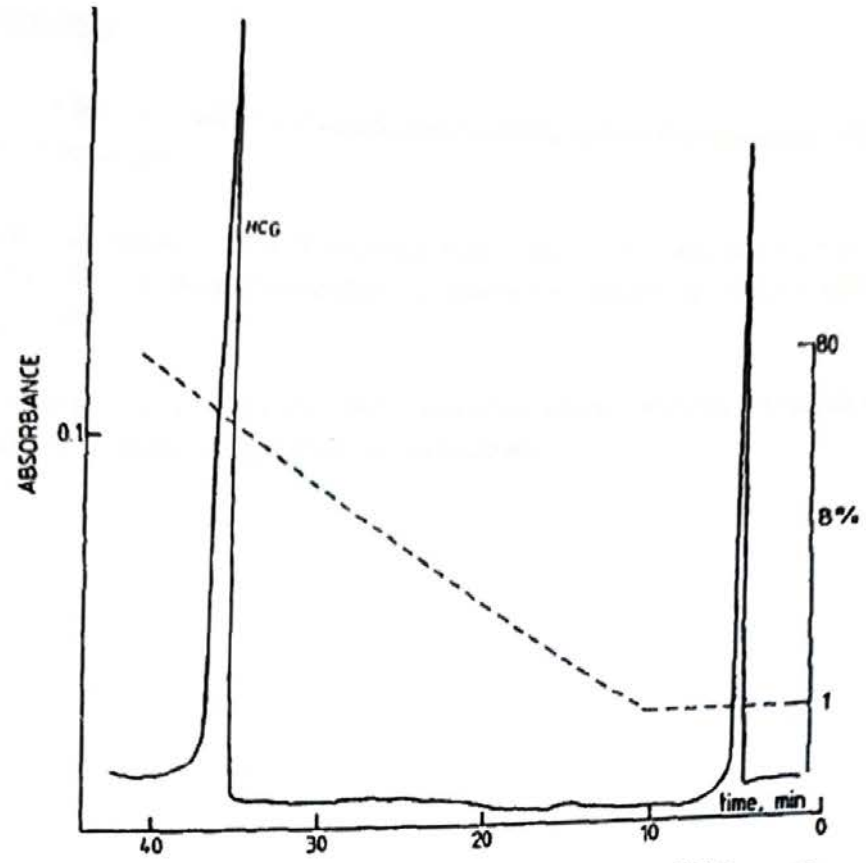
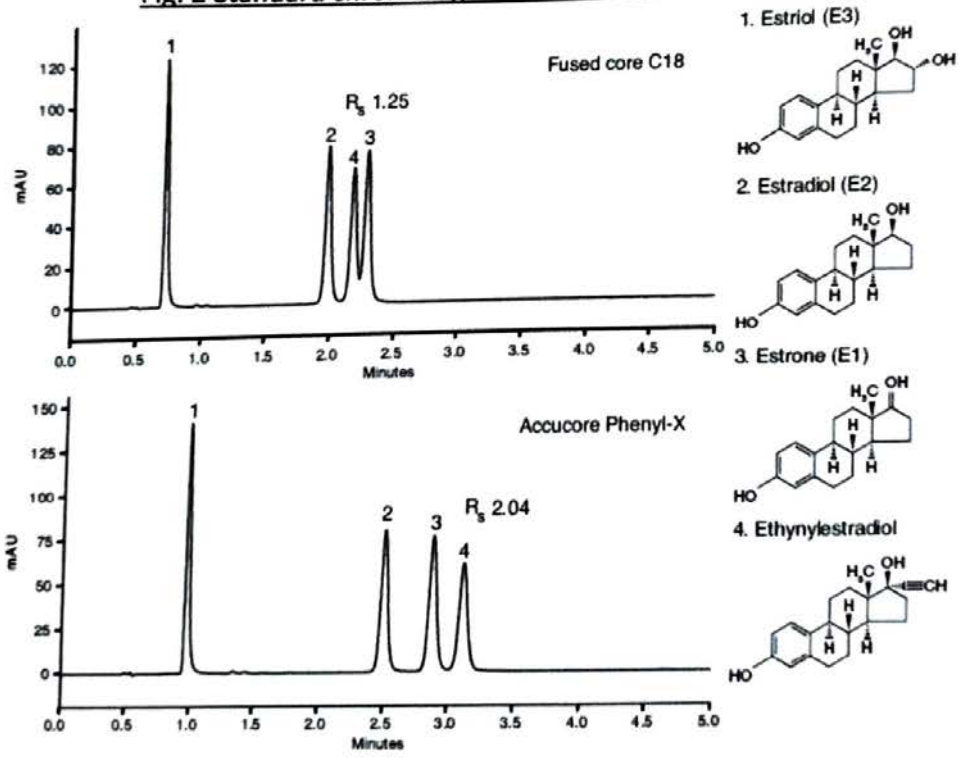


Fig. 2 Standard chromatogram showing separation of Estrogens



List of References

Purification of Human Chorionic Gonadotropin Hormone by Anion Excahnge High performance Liquid Chromatography,

World Health organization (WHO) Technical Report Series, No. 800, Annex 2 of WHO Technical Report Series, No. 927 Recommendations to assure the Quality, Safety and Efficacy of tetanus vaccines (adsorbed)

Pharmacophore 2011 vol. 2 (3) 186 -194, Qualitative and Quantitative Analysis of Various Constituents of Vaccines using Analytical Techniques