

# Original Research Article

## ASSESSMENT OF THE PREVALENCE OF *Chlamydia trachomatis* INFECTION IN DONATED BLOOD AT REGIONAL BLOOD TRANSFUSION CENTRE, ELDORET, KENYA.

### ABSTRACT

**Aim:** To detect *Chlamydia trachomatis* in donated blood at Regional Blood Transfusion center, Eldoret.

**Methodology:** A random selection blood sample was done for this study for a period of one month from July 24<sup>th</sup> 2017 to August 24<sup>th</sup> 2017. Blood bags were numbered 1-87 and picked amongst unnumbered blood bags by each donor in order of donation. The samples obtained were subjected to centrifugation and serum used in the immunoassay test by using the *Chlamydia trachomatis* IgG/IgM Elisa test kit.

**Result:** This study showed that *C. trachomatis* was present in 8% (7 samples) of the blood samples analyzed and 92% were found to be negative.

**Conclusion:** With 8% of the samples testing positive for *Chlamydia trachomatis* this indicated that some of the blood donated to the Regional Transfusion Center is contaminated.

**Keywords:** Blood, *Chlamydia trachomatis*, Detection, Kenya, Transfusion

### 1. INTRODUCTION

*Chlamydia* is a genus of pathogenic bacteria that are obligate intracellular parasites. Chlamydia infections are the most common bacterial sexually transmitted diseases in humans and are the leading cause of infectious blindness worldwide [1]. *Chlamydia trachomatis*, commonly known as chlamydia, is one of four bacterial species in the genus Chlamydia.

*C. trachomatis* is globally a major cause for sexually transmitted diseases (STDs) including Chlamydia. Chlamydia is one of the most common sexually transmitted infections worldwide affecting about 4.2% of women and 2.7% of men [2]. *C. trachomatis* cause various infections that include trachoma (conjunctivitis which is the most reported reason for blindness globally), pneumonic infections, and genital infections in people of both sexes and conjunctivitis in newborns. It is estimated that there are over 50 million new cases of *C. trachomatis* infection annually [3]. However another study done in 2015 showed that about 61 million new cases occurred globally [4].

*C. trachomatis* causes various contaminations among among which is donated blood. A study estimated that about 92 million blood donations are collected per year in different parts of the world [5]. In addition, WHO published that 39 countries in the world still do not routinely screen for transfusion-transmissible infections (TTIs) including HIV, Hepatitis B, Hepatitis C and syphilis [6]. About 47% of blood donations in low-income countries are screened in laboratories which do not have capacity for *C. trachomatis* contamination [7]. Blood transfusion centers are expected to provide blood

38 that is free of contaminants. However, without this there is likeliness of transmitting fatal agents like *C.*  
39 *trachomatis* that may result in deadly diseases that may be found in blood [8].

40 Weström [9] asserted that one of the factors that may contribute to unnoticed *C. trachomatis*  
41 contamination of donated blood is that its infections are to a bigger percentage asymptomatic. This  
42 has commonly been reported in both males and females with about 50% males and 75% females of  
43 those affected having no symptoms.

## 44 **2. MATERIALS AND METHODS**

### 45 **2.1. Sample Collection**

46 Blood samples used in the study were collected aseptically using venipuncture techniques by  
47 qualified personnel of the center following the Pendergraph procedure of phlebotomy [10].

48 Use of aseptic techniques were observed to preserve the integrity of the specimen. Blood samples  
49 obtained were labelled with the donors code for *C. trachomatis* test. The sample blood obtained was  
50 subjected to centrifugation to separate the serum from the other blood components. The serum  
51 samples were then refrigerated at 2<sup>o</sup>-8<sup>o</sup>C in RBTC Hematology Laboratory to be used in the  
52 immunoassay test by using the *Chlamydia trachomatis* IgG/IgM Elisa test kit manufactured by Vircell  
53 Company Spain to find out the presence of *C. trachomatis* in donated blood using the ELISA  
54 procedure as shown below.

### 55 **2.2. Sample Processing**

56 10ml of blood sample from each donor was centrifuged at 1600rpm for 15 minutes. This was done  
57 when clotting had occurred and within 1 hour after blood sample was drawn. Serum was then used for  
58 the indirect immunoassay test.

#### 59 **2.2.1. Indirect Immunoassay**

##### 60 **Preparations and Assay Procedure**

61 The washing solution was prepared in advance. This was done by using distilled water to fill 50 ml of  
62 20x washing solution up to 1000ml after which the solution was warmed up to 37°C in the washing  
63 concentrate during storage before diluting. Once diluted, it was stored at 4°C.

##### 64 **The Indirect Immunoassay Procedure**

65 The incubator was set at 37°C and all the reagents brought to room temperature before use  
66 (approximately 1hour), without removing the plate from the bag. The components were then shaken  
67 well.

68 The plate was removed from the package and the numbers of wells to be employed determined that  
69 is: Four wells for the controls; two for the cut off serum and one each for the negative and positive  
70 sera. Wells not required for the test were returned to the pouch and sealed.

71 For IgG test, 100 µl of serum diluent **2** was added to all wells followed by 5 µl of each sample, 5 µl of  
72 positive control **3G**, 5 µl of cut off control **4G** (in duplicate) and 5 µl of negative control **5G** into the  
73 corresponding wells. The plates were then shaken in a plate shaker for 2 min in order to achieve a  
74 homogenous mixture of the reagents. A sealing sheet was then used to cover the wells and incubated  
75 at 37°C for 45 min.

76 For IgM test, 25 µl of VIRCELL IgG sorbent was added to each of the required wells, except for the  
77 wells where controls will be dispensed. 5 µl of sample was added and then 75 µl of the serum diluent  
78 **2** to each well. Control wells were prepared by adding first 100 µl of the serum diluent **2** to each well  
79 and then 5 µl of the positive control **3M**, 5 µl of the cut off control **4M** (in duplicate) and 5 µl of the  
80 negative control **5M** to the corresponding wells. This was followed by plate shaking in a plate shaker  
81 for 2 min in order to achieve a homogenous mixture of the reagents. A sealing sheet was then used to  
82 cover the wells and incubated at 37°C for 45 min.

83 After incubation, the seal was removed, liquid aspirated from all wells and washed five times with 0.3  
 84 ml of washing solution **9** per well and any remaining liquid was drained off. 100 µl of IgG conjugate  
 85 solution **6G** or IgM conjugate solution **6M** was immediately added into each well and a sealing sheet  
 86 used to cover followed by incubation at 37°C for 30 minutes.

87 After 30mins the seal was removed, liquid aspirated from all wells and washed five times with 0.3 ml  
 88 of washing solution **9** per well. Any remaining liquid was drained off and 100 µl of substrate solution **7**  
 89 immediately added into each well followed by incubation at room temperature for 20 minutes  
 90 protected from light. After incubation 50 µl of stopping solution **8** was immediately added into all wells  
 91 and finally readings were carried out within 1 hour of stopping.

### 92 **3. RESULTS**

93 In this study, a greater percentage of the samples were from the donors between 20-29 years of age  
 94 (60.9%), while the least were below 20 years old group. There was a higher proportion of females  
 95 (55.2%) than males (44.8%). Samples from single donors were 64.4% while divorced/separated and  
 96 widowed were both 3.4%. 75% of the samples were from donors who were currently at tertiary  
 97 education level while none was from those who had never attended attained any form of education.  
 98 The highest proportion of the samples was from unemployed donors (73.6%), 11.5% from self-  
 99 employed and 14.5% from employed persons (Table 1).

100 The largest percentage of samples were from donors who felt they were currently in good health  
 101 (97.7%), had no sexual encounters with persons whom they did not know their background (96.6%),  
 102 had irregular sexual partners (85.1).

103 **Table 1. Overall characteristics of blood donors whose samples were analyzed at RBTC,**  
 104 **Eldoret.**

<b>Characteristics</b>	<b>Categories</b>	<b>Total (N=87) No (%)</b>
<b>Age groups</b>	Below 20 years	4 (4.6)
	20-29 years	53(60.9)
	30-39 years	25(28.7)
	40 and above	5(5.7)
<b>Gender</b>	Male	39(44.8)
	Female	48(55.2)
<b>Marital status</b>	Single	56(64.4)
	Married	25(28.7)
	Divorced/Separated	3(3.4)
	Widowed	3(3.4)
<b>Education level</b>	None	0(0)
	Primary	7(8.0)
	Secondary	14(16.1)
	Tertiary	66(75.9)
<b>Employment status</b>	Unemployed	64(73.6)
	Self-employed	10(11.5)

	Employed	13(14.9)
<b>Good health</b>	Yes	85(97.7)
	No	2(2.3)
<b>Sexual activity</b>	Yes	3(3.4)
	No	84(96.6)
<b>Irregular sexual partners</b>	No	13(14.9)
	Yes	74(85.1)

105

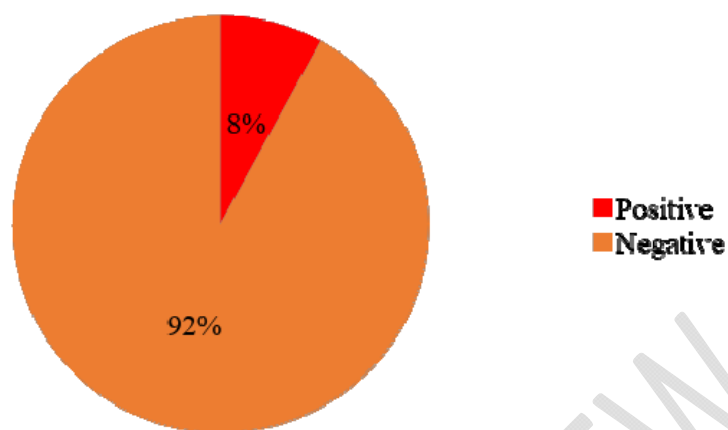
106 **Detection of *Chlamydia trachomatis* in donated blood using IgG/IgM Elisa kit**

107 Samples with indexes below 9 were considered as not having IgG or IgM specific antibodies against  
 108 *C. trachomatis* while samples with indexes above 11 were considered as having IgG or IgM specific  
 109 antibodies against *C. trachomatis*. Therefore this study showed that *C. trachomatis* in blood donors'  
 110 samples at RBTC was found to be present in 8% (7 samples) of the blood samples analyzed at RBTC  
 111 and 92% were found to be negative. (Table 2).

112 **Table 2: Antibodies Present in donor samples indicating *Chlamydia trachomatis* infection**

Week	Donor No.	Antibodies against <i>C. trachomatis</i>	
		IgM Antibodies	IgG Antibodies
1	1-25	-	-
2	32	-	+
	37	-	+
	41	+	+
	48	+	-
3	53	+	-
	59	-	+
4	86	-	+

113 - =Absence of antibodies, + = Presence of antibodies



114

115

**Figure 1: Proportion of donors' samples with Chlamydia trachomatis at RBTC**

116

**Associations Between *C. trachomatis* and Selected Variables**

117

With the significance level at 0.05 the statistics revealed that having irregular sexual partners dependency with occurrence of *C. trachomatis*  $\chi^2 (1) = 4.667$ ,  $p=0.031$  but not significant. (Table 3)

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119

120

**Table 3: Detection of *C. trachomatis* infection in donated blood with respect to risk factors at RBTC, Kenya.**

121

Characteristics		<b>C. <i>trachomatis</i> status</b> Positive N=7 Out of 87 samples	<b>Chi Square tests</b>		
			Pearson Chi- Square	Valid cases	Asymp. Sig. (2- sided)
<b>Age groups</b>	Below 30 years	4	0.236	87	0.627
	30 years and above	3			
<b>Gender</b>	Male	2	0.706	87	0.401
	Female	5			
<b>Marital status</b>	Single	4	0.173	87	0.677
	Non-singles	3			
<b>Education level</b>	Primary and below	1	0.401	87	0.527
	Secondary and above	6			
<b>Employment status</b>	Unemployed	4	1.055	87	0.304
	Self- employed and/	3			

	Employed				
<b>Irregular sexual partners</b>	No	3	4.667	87	<b>0.031*</b>
	Yes	4			

122 **Note:** Non-singles include married, divorced, separated or widowed

123 \* Significant at  $p < 0.05$ .

#### 124 4. DISCUSSION

125 The presence of *C. trachomatis* in blood samples was determined based upon the reaction of  
 126 antibodies in the donor sample tested with the antigen adsorbed on the polystyrene surface. Unbound  
 127 immunoglobulins were washed off, this was then followed by an enzyme anti-human globulin binding  
 128 the antigen-antibody complex in a second step. After a new washing step, bound conjugate was  
 129 developed with the aid of a substrate solution (TMB) to render a blue colored soluble product which  
 130 turned into yellow after adding the acid stopping solution [11]. IgG/IgM antibody detection are markers  
 131 for a Chlamydia-positive immune response, either for current, chronic or past infections. The assay  
 132 used in this study was the COMP (Complexes of Outer Membrane Proteins) of *C. trachomatis*, free  
 133 from LPS which is responsible for most cross-reaction with other Chlamydia species. ELISA yields  
 134 very good agreement with the target results (IgM100%, IgG 98%) in quality assessment schemes  
 135 [12]. It provides good agreement with characterized samples from quality assessment schemes of  
 136 IgM 100%, IgG 98% [12]. Therefore ELISA technique is paramount for detection of *C. trachomatis*  
 137 antibodies.

138

139 Occurrence of IgM antibodies without IgG being present is an indication of primary infection while  
 140 presence of IgG antibodies without IgM antibodies in the blood sample is an indication of re-infection  
 141 or presence of previous infections since IgG antibodies persists in the body for a long period of time.  
 142 Presence of both IgG and IgM antibodies in the sample shows an ongoing infection.

143 In a study from Makkah Saudi Arabia, 8.7% of the women were positive for IgG antibodies to *C.*  
 144 *trachomatis* with antibody indexes of 1.4–2.0 [13]. Low prevalence in Saudi patient population may be  
 145 due to the adherence of strict moral principles and code of ethics in Saudi Arabia. In comparison to  
 146 this study, 71.4% of women were positive for both IgG/IgM antibodies which is much higher.

147 Similarly, it has been shown in the USA and Europe that demographic factors which increase the risk  
 148 of chlamydial infection include youth, single marital status and multiple sexual partners [14].  
 149 Therefore, a closer attempt should be made to correlate risk factors and disease entity when  
 150 screening for *C. trachomatis*.

151 According to a study done by CDC,[15] on the prevalence of *C. trachomatis* among the adults, the  
 152 highest rates is between 20 to 24 years old, however the age-specific rates for women was as low as  
 153 to 16 to 26 years old. This current study however slightly differs from the statistics by CDC. The age  
 154 between 20 to 29 years of age showed the highest prevalence.

155 Multiple partnerships may increase the likelihood of encountering a sexually transmitted pathogen  
 156 through the increased probability of choosing a partner with infection, while having new or casual  
 157 sexual contacts may be related to increased risk because of a reduced familiarity between partners  
 158 [16]. Statistics from this study revealed that having irregular sexual partners might have had  
 159 dependency with occurrence of *C. trachomatis*  $X^2 (1) = 4.667$ ,  $p=0.031$ . Therefore this study concurs  
 160 with the later however it is not conclusive as per the statistics on the *chi square* on table 3.

161 Younger age has shown consistently to be associated with increased risk of chlamydial infection  
 162 among the sexually active population with the highest incidence rates of infection being reported  
 163 consistently in adolescents and young adults in Canada and the United States [17]. This study

164 confirms the same as the highest prevalence is between the ages of 20 to 29. There are a number of  
165 reasons why adolescents are at greater risk for genital chlamydial infection than older people. A  
166 higher risk in adolescent females may be associated with certain aspects of physical development  
167 that make this group more vulnerable to sexually transmitted infections, including the persistence of  
168 columnar epithelium on the cervix, which supports the growth of *C trachomatis*, and changes in  
169 vaginal flora and mucus production [18]. As well, the older may have acquired partial immunity after  
170 initial or serial infections in the past [18].

## 171 **5. CONCLUSION**

172 The findings of this study therefore indicates that some blood donated to the Regional Transfusion  
173 Center was contaminated with *Chlamydia trachomatis* with 8% of the total samples indicating positive  
174 for *C. trachomatis* infection while 92 % were negative. Moreover the age group of between 20 to 29  
175 years old had the greatest percentage of those with *C. trachomatis*. Having multiple irregular number  
176 of sexual partners increases the chances of having *Chlamydia trachomatis* infection. However this is  
177 not be conclusive according to the statistics of this study.

178 The sex partners of donors with Chlamydial infection should therefore be referred for medical care  
179 through the efforts of individual donors. A further study is also recommended on the exact level of *C.*  
180 *trachomatis* by use of absorbance ranges.

## 181 **6. COMPETING INTERESTS**

182 "The authors declares that they have no competing interests"

## 183 **7. ACKNOWLEDGMENTS**

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## 186 **8. AUTHORS' CONTRIBUTIONS**

187 The authors' responsibilities were as follows; CP was author of the script, RF was the first supervisor  
188 while FK was the second supervisor.

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