Journal of Medicine and Biology

Review Article | Vol 3 Iss 1

Shiga-Toxin Producing *Escherichia coli*: Pathogenesis, Occurrence (Prevalence), Screening Methods, and Anti-STEC Antibody Development for Immunodiagnostic Assay Design

Biniam Moges[1*], Temesgen Assefa[1], Mesifen Dagne[2] and Degisew Yinur[3]

[1]*Department of Biotechnology, Debre Berhan University, Amhara, Ethiopia*

[2]*Center of Food Science and Nutrition, Addis Ababa University, Addis Ababa, Ethiopia*

[3]*Department of Biotechnology, Wolkite University, Guraghe, Ethiopia*

Correspondence should be addressed to Biniam Moges Eskeziyaw, biniammoges4@mail.com

Received: May 25, 2020; Accepted: June 15, 2020; Published: June 22, 2020

ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) are the leading pathogenic strain that leads severe human diseases ranging from diarrhea to hemolytic uremic syndrome and death by produced one or more shiga toxins (stx_1, stx_2, or their virulence variants), which inhibits protein synthesis of infected host cells, thus leading to cell death. According to WHO, (2019) report, diarrheal disease due to STEC and other pathogenic *E. coli* strain infection are the most frequent and the second leading causes of death globally. Over the years, many screening techniques to aid in the diagnosis of STEC strain have been developed; yet, many of the available diagnostic assays are time consumed, require expensive materials, trained personnel and also may not be readily available in many areas. Recently the emerging of new STEC strain, non STEC bacteria and virus like COVID-19 has come up for the need of designing very rapid and mass testing approaches that used to diagnosis with rapidly, sensitively, specifically, low cost and accessible. Henceforth, this review will play a vital role on rethinking on how researchers and countries how to deal such a crisis and how to reorganize research institutions, also how to reorient the health system at large on how to curb problems with respect to designing of a new diagnostic kit and create a potential capacity for fast and cost effective testing mechanisms.

KEYWORDS

Antibody; Diagnosis; Shiga toxin producing *E. coli*; Pathogenicity; Immunodiagnostic assay

INTRODUCTION

Escherichia coli is a gram-negative, rod shaped flagellated bacterium under the family of *Enterobacteriaceae*. Over 125 years later, *E. coli* was acquired as commensals, harmless and non-pathogenic strains [1]. However, some of them turn out to be pathogenic through the acquisition of multiple virulence determinants genes [2].This pathogenicity of *E. coli* is developed by getting virulence factor coding foreign genes from pathogenic bacterial strains through horizontal gene transfer process [3].These virulence

Citation: Biniam Moges, Shiga-Toxin Producing Escherichia coli: Pathogenesis, Occurrence (Prevalence), Screening Methods, and Anti-STEC Antibody Development for Immunodiagnostic Assay Design. J Med Biol 3(1): 1-20.

genes present either integrated into the chromosome or self-replication within the new host that leads to provide highly diverse novel adapted pathogen [4]. Currently, there are six pathotypes of *E. coli* are commonly recognized as diarrheagenic strain [5,6]. Among these, Stx producing *E. coli* (STEC) strain are the leading pathogenic strain that leads to severe human diseases ranging from diarrhea to hemolytic uremic syndrome and death of humans in worldwide [7] by producing one or more Shiga like toxins (stx_1, stx_2, or their subtype virulence variants), which prevents protein synthesis of infected host cells, thus leading to cell death [8]. STEC strains, in addition to their stx, also have Lipopolysaccharide (LPS) (endotoxin), as a major component and immunogenic or virulence factors. It contains three main distinctive regions such as lipid A, core oligosaccharide, and O-antigens [9]. Among these O-antigens are highly immunogenic and most variable bacterial cell constituents that contribute to O- antigen diversity and ideal to use as antigen source for serotype diversity and developing immunodiagnostic assay [10]. Certain strains of *E. coli*, like, O26:H11, O45:H28, O91:H21, O103:H2, O104:H4, O111: H-, O113:H21, O121:H19, 0128: H11, O145:H28 and, O157:H7 are the most potential lethal toxin produced strains and leads to diarrhea or illness [11].

According to [12]; study yearly around 2,801,000 cases of acute illnesses; from those 3,890 cases of HUS developed and 230 deaths were reported globally due to STEC infection. Among those, above 10,200 cases of STEC infections occur in Africa annually. Over the years, many diagnostic assays (from stool culture to molecular gene level) have been developed to aid for the diagnosis of STEC strain and they are available commercially; however, they have different limitations such as time-consuming, less specificity and sensitivity, require expensive equipment and trained personnel and also may not be readily available in many countries [13]. Developing countries including Africa STEC strains detection is frequently time consuming and incomplete [14], that leads to potential misdiagnoses/ mistreatments and costing billions of dollars for medical care [15].

Therefore, to eliminate or eradicate this global health problem by applying effective treatment and prevention of STEC infections, different researches recommendsdesigning a new detection method approaches that can detect all STEC strains with simply, rapidly, sensitively, specifically and less cost are necessary [16]. In general this review will have a pivotal role in curbing problems related to testing of fast, reliable highly and cost effective diagnostic mechanisms for countries; and also may come up with a new approach for the world that needs mass testing especially at this critical time where the word is facing an infectious and very contagious disease like COVID-19; of course rampant testing is crucial so as to mitigate the problem.

PATHOGENICITY MECHANISM OF STEC

Shiga toxin producing E coli is one of the outbreak pathogenic bacteria strain through the acquisition of multiple virulence determinants and mobile genetic elements such as plasmids, bacteriophages (Stx), transposons and pathogenicity islands (LEE) as indicted in (Figure 1) [5,17].

The pathogenicity mechanism of STEC strains are a multi-step process involving different complex interactions between STEC bacteria and host cell. STEC strain have ability to resist acidic environments of the stomach that means gastric acid in the stomach does not totally remove of this pathogenic bacteria that leads to conducive condition to the infection process of the host cell. The pathogenicity mechanism of STEC strains in host cell is mediated through genes coding shiga toxins (Stx), genes existing on the pathogenicity island in the locus of enterocyte effacement (LEE), and the presence other virulence factors coding genes. Among the main

virulence factors endorsed to STEC disease pathogenesis is a phage-encoded shiga toxin (Stxs) [18].

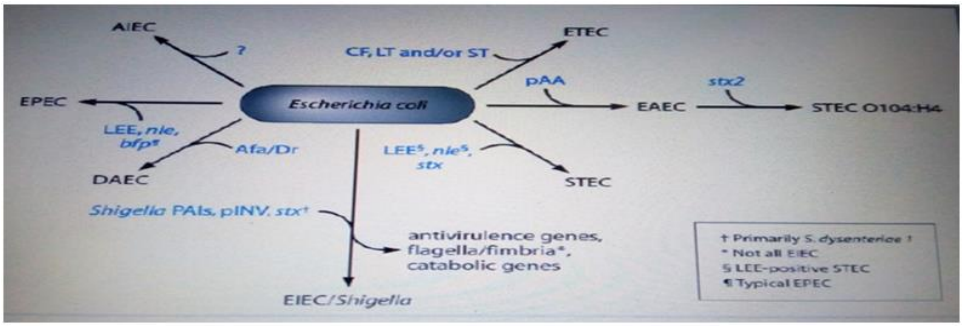

Figure 1: General overview of pathogenic gene acquisition and loss for different pathotypes.

In the outer membrane part, STEC strain also has Lipopolysaccharide (LPS) as a virulence factor. It comprises of three different regions: lipid A (main driver of inflammatory responses), core oligosaccharide, and polysaccharides (O-antigens).

Gene	Function
EaeA	intimin
Saa	Self-binding adhesion protein in LEE-negative strains.
Stx1	Toxin Stx1
Stx2	Toxin Stx2
Stx2b	Stx2b toxin subtype
Stx2c	Stx2c toxin subtype
Stx2d	Stx2d toxin subtype
Stx2eS	tx2etoxin subtype
Stx2f	Stx2f toxin subtype
flich7	Flagellum H7
rrb E	Synthesis of the O anitgen for O157
wzxO45	
wzxO103	Synthesis of the O anitgen for O103
wbqEO121	Synthesis of the O anitgen for O121
wbqFO121	
wzxO145	Synthesis of the O anitgen for O145
wzxO26	Synthesis of the O anitgen for O26
wzxO111	Synthesis of the O anitgen for O111
uidA	B-Glucoronidade
hly933	Enterohemolysin in the human O157:H7 serotype
hly21	Enterohemolysin allele incattle O157:H7 serotype
ehx A	Enterohemolysin
Esp P	Extracellular serien protease produced by O157:H7

Table 1: Pathogenic genes of interest in STEC strains and their expression in the cells.

O-antigens are highly immunogenic and variable within bacterial cell constituents, due to difference in the types of sugars present, arrangement in the O-unit, and linkages between O-units that contribute to O- antigen diversity. This character of LPS used as a basis for serotype diversity; there variation is not only happen between different bacterial species, but also between individual clones within a single species, therefore, they are targeted by the adaptive immune system [8]. This high variability is important characteristic for researchers to tracing infection root and risk assessment of STEC isolated from patients or food for human consumption based on their serotype [10]. The most studied currently recognized genes that code the pathogenicity of STEC strain are described in (Table 1) [13].

The infection mechanism of action of the STEC strain initiates by attaching of the STEC strain to the target host cell. After adhered to intestinal epithelial cells, STEC strain begins to produces and releases stx1, stx 2, both, or subtype stx1 (stx1a, stx1c, and stx1d) and stx2 (stx2a, stx2b, stx2c, stx2d, stx2e, stx2f, and stx2g) into extracellular milieu [19]. The produced shiga toxins pass across the intestinal epithelium and delivers to its receptor which is Globotriaosylceramide (Gb3), by circulating neutrophils. In this cause the B-subunit which is responsible for the binding of the toxin to host cell Gb3 receptor which leading to the internalization of the toxin. On the other hand, different study reports showed that Stx also can enter Gb3 negative cells through binding to Globotetraosylceramide (Gb4) surface glycolipid receptors. This indicates that STEC strains can deliver stx toxin through Gb3 and Gb4 receptor into host cell and cause to disease and cell death [20].

Shiga toxin of STEC strain is formed via a subunit A, once produced it is internalized in to host cell and transported to the Golgi complex and endoplasmic reticulum. This toxin exerts toxic action and enzymatically active N-glycosidase which inhibits protein production of the host cells via degrading of adenine base specifically 28SrRNA component of the eukaryotic ribosomal 60S subunit, causing cell death and or apoptosis (Figure 2) [13,21].

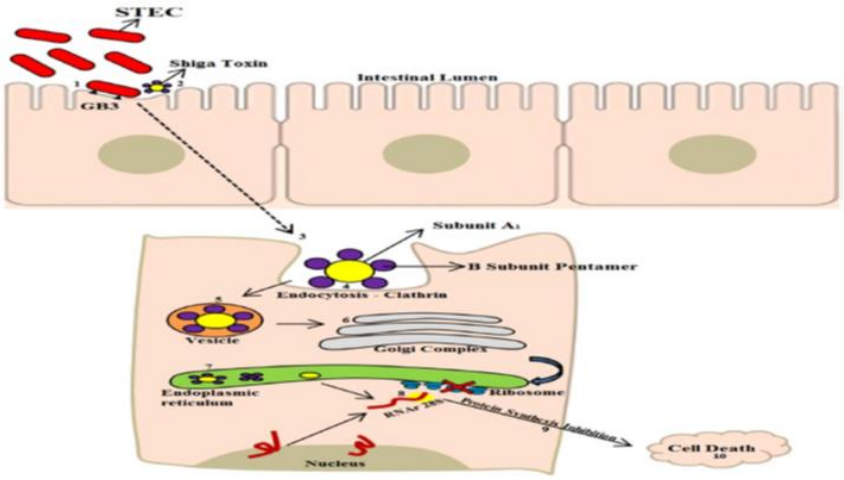

Figure 2: Pathogenic mechanism of action of the shiga toxin in host cell.

HOST IMMUNE RESPONSE AGAINST STEC STRAIN INFECTION

The general process of STEC to infect host cell can be described as follows: i) colonization of the gut, ii) virulence factors effect on the host cell and iii) disease caused by the virulence factors. Therefore the host cell designed their defense mechanism via develop specific cellular and humeral immune responses by targeting peripheral blood and intraepithelial lymphocytes (IEL). Once the STEC strain attached to host cell surface, the innate (natural/ non clonal/ non adaptive) immune system of host cell defense against STEC infection is activated as first phase of defense using their pathogen pattern recognition receptors (Toll-like receptors (TLRs) [22]. At this phase of defense the pathogens are limited spread through the host's physiology: Skin pH or proteases in the saliva by penetrate the host cell physical barriers (skin, mucous membranes and enzymes). When STEC strain recognized by phagocytic cells through pathogen-associated molecular patterns, leading to intracellular signaling for the production of cytokines and chemokines which activate adaptive immune system [23]. One of the best-known PAMPs is endotoxin (LPS), which is responsible for enhancing innate host defense system in gram-negative infections [24]. The innate immune system comprises of cells and complement that are induced by microbial structures which is recognized by different pattern recognition receptors (Toll-like receptors -TLRs) of monocytes, macrophages, dendritic cells and neutrophils immune system [25].

In addition to innate immune response, adaptive immune response of the host cell also activated by cytokines and chemokines that are produced during recognition of immunogenic (antigenic) part of STEC strain by TLRs of the host cell [26]. Different studies report indicates that about 10 TLRs have been known in humans and 13 TLRs in mice, among these TLR 4 and TLR 5 recognize specifically lipopolysaccharide and flagellin of Gram-negative bacterial respectively. They are found at the cell surface of phagocytic cells, but may also be recruited into phagosomes [27]. Different signal transductions are involved for immune system activation against STEC infection. One of the most vital signaling cascades that

involve in the activation host immune response system to eliminate STEC pathogen from the cell is interferon-gamma (IFNγ) signal transduction pathway [28]. Macrophages and natural killer secrete cytokines, including IFNγ, which activated T cells following STEC infection resulting in the activation of above 2000 IFNγ-stimulated genes in recipient host cells that together support the host defense against pathogenic microbes [20].

Humeral and cell-mediated adaptive immune responses to STEC infection is generated by lymphocytes when the innate immune response unable to remove the STEC pathogen from the host cell. Cooperation defense between innate and adaptive immunity arises through chemical messengers and through direct contact between cells of the innate and adaptive immune responses (primary and secondary) that leads to an optimum defense against the target pathogens as showed in (Figure 3) [29].

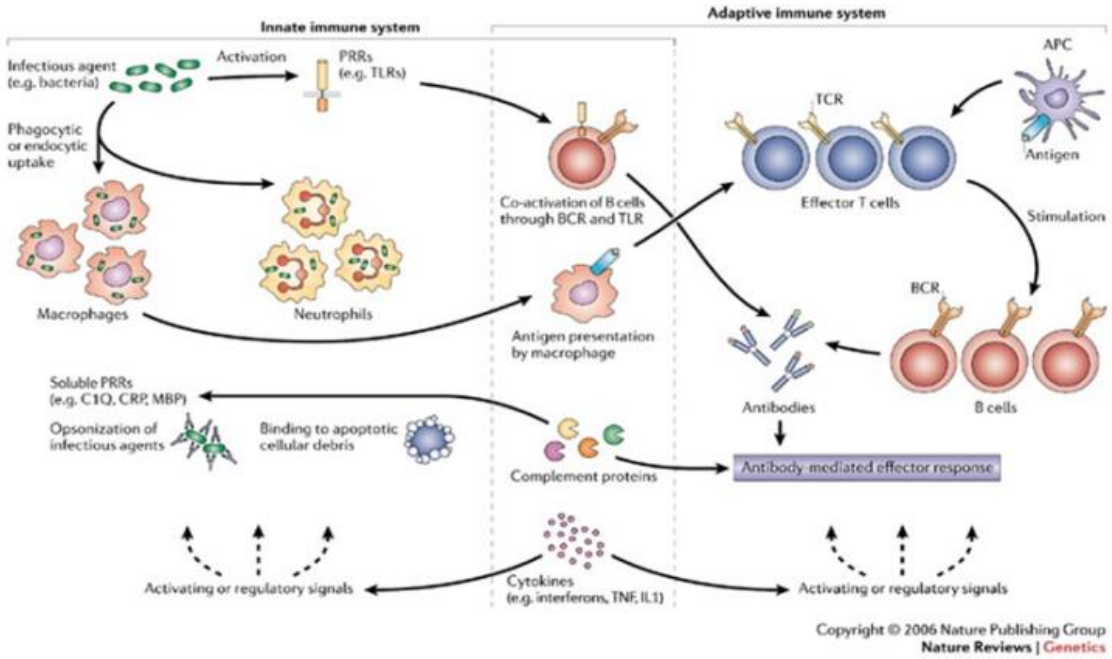

Figure 3: Innate and adaptive immune response against Gram negative bacteria (STEC) Strain.

PREVALENCE OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC)

Shiga toxin-producing *Escherichia coli* (STEC), both O157 and many non-O157 serotypes, are identified as a food-borne bacteria associated with outbreaks worldwide. They cause human serious disease, diarrhea to hemolytic-uremic syndrome (HUS), a life-threatening complication and death. Near to 5% - 10% of people with STEC infection has a chance to develop hemolytic-uremic syndrome (HUS) and will die or have permanent renal failure [17]. STEC is a global problem, and more than 60 serotypes have been recognized with human disease. Globally, STEC causes annually 2,801,000 acute illnesses, with an incidence rate of 43.1 cases per 100,000 persons. From those, 3,890 cases of HUS developed and 230 died. Among those, a total of 10,200 cases of STEC infections occur in Africa with an incidence rate of 1.4 cases per 100,000 people annually. STEC O157:H7 accounts 10% to this burden [12].

Escherichia coli O26, O45, O103, O111, O121, O145, and O157 are the leading shiga toxin-producing *E. coli* (STEC) O- serogroups associated in outbreaks of human foodborne illness worldwide. From these O157:H7STEC is the most prevalent and a serious public health problem HC and HUS in children both developed and developing countries [30]. Due to prevalent in human clinical infection to date, STEC prevalence studies and detection

(culture and molecular) method development and optimized have been concentrated on *E. coli* O157:H with slight attention of the risks posed by non-O157 serogroups [31]. However, some prevalence studies report, O26, O121, O103, O111 and O145 are identified as non-O157 STEC serotype. According to European Union STEC infection reported from 2007 to 2011 were due to O157 (49% to 76%), O26 (7% to 11%), O103 (3% to 4%), O91 (2% to 3%), O145 (2% to 3%), O111 (1% to 2%), and O128 (1%). In US, from 2000 to 2010 infections due to non-O157 serotype increased from 0.12 to 0.95 per 100,000 inhabitants, whereas O157 infections cut down from 2.17 to 0.95 per 100,000 inhabitants [13]. Overall, STEC strain isolates have been reported in developed and developing countries, from humans, animals, food products and the environment. Out of 30 reviewed cases, 10 (33.3%) from human patients and 20 isolations (66.7%) from food stuffs and animals [14]. In Africa all virulence genes, such as *stx1*, *stx2*, *eae* and *ehxA* genes, have been detected in humans, animals, food products and environment. However, stx1+ stx2 are dominant and Cattle are the most common source of STECO157:H7, as shown in (Table 2) [12].

Gene combination	Source	Report	Country
Stx1	Cattle feces, milk	2	Kenya
Stx2	Cattle feces, human stool	2	Kenya
stx1 + stx2	Cattle feces, water, fish, milk, human stool, beef, goat, sediment	6	Ethiopia, Egypt and Morocco
eae + ehx A	Cattle feces, pig	1	South Africa
eae + stx2 + ehx A	Cattle feces, cattle carcass	2	Tanzania and Algeria
stx1 + stx2 + eae	Human stool, beef	2	Cameroon and Morocco
stx1 + stx2 + eae + ehx A	Cattle feces, goat, sheep, pig, human stool	2	Nigeria, Tunisia and South Africa

Table 2: STEC virulence factor combinations from studies in Africa.

DIAGNOSIS/DETECTION MECHANISM OF STEC STRAIN

There are a number of complications associated with the screening of STEC infection due to it number flexibility, large numbers of STEC at the early stages of infection and dramatically dropping of its number as the disease progresses. In addition, the diversity of shiga toxins in nature and their broad range of host cell, screening and differentiation of toxins or outer membrane of O157:H7 and other new and emerging serotypes of non-O157 (O26, O45, O103, O111, O121, and O145) [32] by using very rapid, prompt, accurate, specific, sensitive and require minimal specimen volumes is important for effective and timely outbreak responses and prevent pointless invasive and expensive investigative procedures or administration of antibiotic therapy [2].

Historically extensive progress has been made in the development of screening assays for STEC strains detection and identification based on the occurrence of Stxs and their immunogenic outer membrane (LPS) parts with differs in complexity, speed, sensitivity, specificity and cost in human, animals and environmental samples [3,13]. Some of them are stool culturing and isolation based on inability to ferment sorbitol on sorbitol-containing agar, polymerase chain reaction (PCR) using a multiplex system, which is based on many types and subtypes of Stx at the same time, quantitative real-time PCR (qPCR), which measure the presence of a given gene in a sample. Reverse Transcription qPCR (RT-qPCR), which measures gene expression, other method has been developed using antibodies based on their immunological properties. However each diagnostic or

detection methods have their own advantages and limitations [34].

The main limitation of these developed detection methods are time requirement (24 hours - 48 hours for culture), require high specimen volumes, difficulty in isolating DNA from sample (for PCR) and low sensitivity (for immunodiagnostic) [35]. Generally an ideal detection or diagnostic method fulfill five principal requirements such as highly specific (detecting only the target bacterium), high sensitivity (detecting as low as a single live bacterial cell), short time-to-results, simplicity and cost effectiveness. For example, culture takes long time to give the results. Whereas, PCR, antibody-based and biosensors techniques provide result within short time, but require expensive reagents and sophisticated equipment which make the method expensive [36].

CULTURE BASED METHODS FOR DETECTION OF SHIGA TOXIN-PRODUCING *E. COLI*

For a long period time, Sorbitol-MacConkey Agar Culture (SMAC) detection method has been the most commonly used for identification of STEC strain mainly in Northern America and Europe and O157 and O157:H2 were detected predominantly [37]. STEC O157:H2 *E.coli* can be easily identified from other commensal *E. coli* strain by using their inability to ferment sorbitol within 24 hours of incubation at 35°C on Sorbitol-MacConkey agar (SMAC), cefiximetellurite-sorbitol MacConkey agar (CT-SMAC), or CHROMagarO157. Principally, most *E. coli* strain ferments sorbitol within 24 hours and generate β-glucuronidase [38]. Whereas, O157:H7 dominant STEC serotype species do not ferment sorbitol and not produce β-glucuronidase and also colourless on SMAC or CT-SMAC [39] but it generate pink colour on CHROMagarO157 [40]. CT-SMAC and CHROMagarO157 mediums are the most selective than SMAC, which enhance the sensitivity of culture for screening of O157 STEC serotype. SMAC medium is limited to screen 0157: H STEC serotype from other background organisms on the plate and it may generate false positive results because of the emerging serotypes of sorbitol fermenting non-O157 and O157 STEC. However, this limitation can be overcome by the use of chromogenic medium which increase the specificity and sensitivity of SMAC for STEC identification [39]. Representative STEC culture test results on various culture mediums are showed in (Figure 4) and ddifferent type's enrichment and culture medium and methodologies used to detect STEC are indicated on (Table 3) [13].

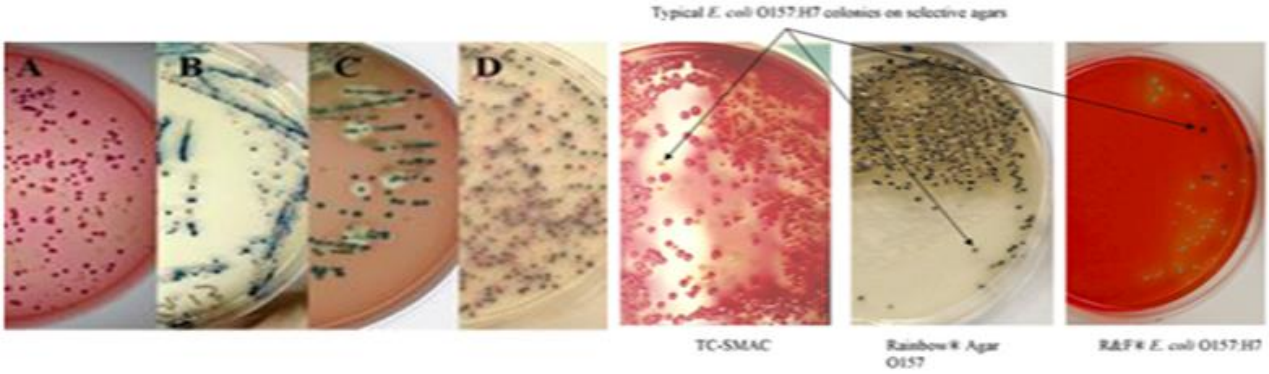

Figure 4: STEC isolation on different selective media. (A) STEC growth isolate on CT-SMAC, (B) STEC colonies look like as pale on CT-SMAC and blue on NT-Rainbow Medium, and non-O157STECs appear as pink colonies on NT-Rainbow, (C) STECS producing β-galactosidase and hemolysin on sheeps blood agar which showed by blue colonies with a zone of clearing and (D) non-O157STECs growing as blue colonies on CHROMagar [35].

Sample	Enrichment	Culture media	Genes	Serotype
Foods	mBPWp	L-EMB, SHIBAM	Stx1, Stx2, uidA, O serogroups	O157:H7
Foods	mTSB IMS	mRBA, SBA	Stx, eae, O serogroups	O26, O45, O103, O111, O121, O145
Foods	mTSB or BPW	TBX/Other suitable	Stx, eae, rsbE, wbdl, wzx, ihp1, wzx	O26, O103, O111, O145, O157
Fecal sample	mTSB	CT-SMAC, CHOMagarTM	Stx1, Stx2, eaeA	General STEC
Rectal stool grab	mEC	MAC	Stx1, Stx2, bfp, EAF plasmid, hlyA, eae, espA, espB, espD, tir	General STEC
Fecal, plant, soil	TSB	CT-SMAC, NT-RA, mSBA	Stx1, Stx2abc, Stx2ex, Stx2f, ompA	General STEC
Raw meat, raw milk and juice	mTSB	CCV-TBX, Luria, Agar	Stx1, Stx2, rfbEO157	General STEC
Ground beef	TSB	wSBAm, SMAC-BCIG	Stx1, Stx2, eae, ehx	General STEC

Table 3: Different types of medium and methodologies used to detect STEC.

However, some studies were conducted in 2013 and their result indicated that O157:H7 strains able to fermenting sorbitol and recognized as an outbreak of a sorbitol-positive O157:H7 strain. This investigation indicates the constraint of screening using bacterial metabolism character alone [41]. Culture-based methods (e.g. sorbitol-MacConkeyagar) remain the gold standard test, cost-effective and panorama to identify viable bacterial isolates for typing. For this reason, there has been increased progress and use of agars which also select for non-O157STEC [42]. Yet, the biggest drawback in the culture techniques are tedious, time consuming, and low sensitive, false negative results due to emerging serotypes of non-157 STEC and sorbitol fermenting O157:H-STEC limit the utility [43].

TISSUE CULTURE CYTOTOXICITY ASSAYS (VCA) FOR DETECTION OF STEC

The Vero cell (derived from African green monkey kidney) and HeLa cell lines (lacks Gb4- less sensitive to Stx2e) have been developed to detect Shiga toxins since they have high concentrations of globotriaosylceramide Gb3 and Gb4 (target receptor for Stx2e) receptors for STEC (stxs) due to that they are very sensitive to shiga toxin and use the toxins to entre in to eukaryotic cells [44]. The Vero cells assay is implemented by transfer of STE cell-free supernatants to tissue culture monolayer's of Vero cells for incubation and observed their typical cytopathic effect. In this cause the incidence of Stxs, Vero cells take a round shape and disconnect from one another and the degree of cytotoxicity can be estimated within 24 and 48 h by using microscope but the cytotoxicity effect may be due to other toxin or bacterial product, therefore it is important to use a neutralization assay (which avoid VCA specificity problem) with specific antiserum(anti-Stx 1 and Stx 2 antibodies) to confirm that the cytopathic effect is because of the generation of Shiga toxins [35]. Many studies have shown that the Vero cells assay is very sensitive, However, because of several disadvantage have limited its routine used in most clinical diagnostic laboratories because it requires a highly skilled technician with tissue culture technique to maintain the cell lines as well as antibodies for validation of shiga toxin, the availability of cell monolayer's and specific antibodies, requires cell culture facilities and time consuming to obtain the results 48 hours - 72 hours [45]. To avoid the limitation concern with tissue culture cytotoxicity assays, non-culture assays that can screen shiga toxins generated by STEC have been first announced in the United States in 1995. This assay can detect all serotypes of STEC with quickly to obtain the results than culture [32].

IMMUNOLOGICAL METHODS FOR STEC STRAIN DETECTION

The first immunoassay was developed by Rosalyn Sussman Yalow and Solomon Berson in the 1950s. Immunological screening of STEC strains are principally based on the binding of antibody-antigen interactions, whereby a specific antibody will bind to its specific antigen immobilized on membranes and horse radish peroxidase for detection or using mAb or pAb antiserum against shiga toxin produced by STEC strain after an enrichment step [46]. Polyclonal antibody is more important to develop a better assay than monoclonal antibody because pAb (multiple epitopes to react with antigen) have higher reactivity than mAbs [8]. Immunological screening technique is the most a widely used method to identify STEC antigen in bacterial cultures, food samples and stool samples. Currently, several different types of commercialized immunological diagnostic assay kit with different setups have been developed for the detection of Stx1 and Stx2 producing E.coli and other pathogenic antigen/stx. Such as immunopreciptate, immunodiffusion, electroimmuno diffusion, Radioimmunoassay (RIA), Counting Immunoassay (CIA), Enzyme-linked immunosorbent assays (ELISA), Immuno agglutination, latex agglutination fluoroimmnoassay (FIA), Chemi-luminescence Immunoassay (CLIA) and others [47].

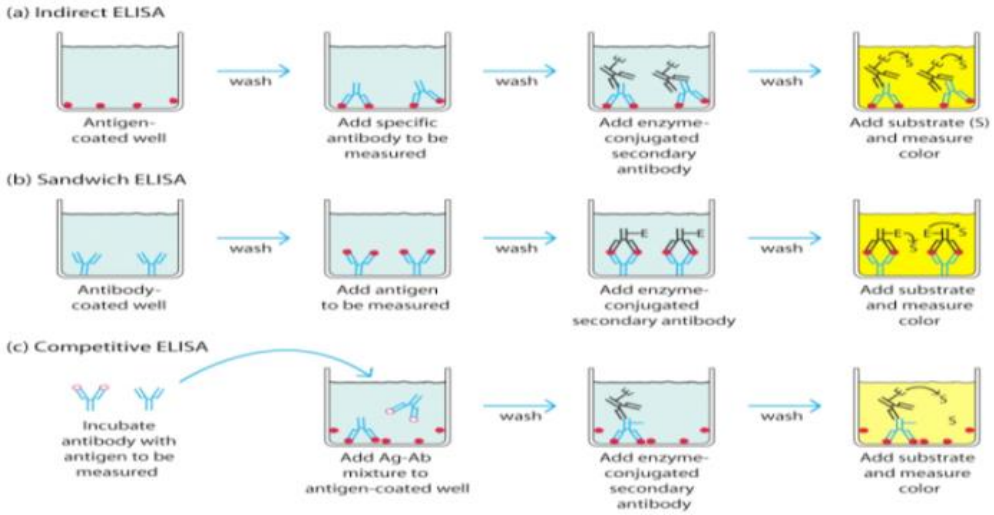

Figure 5: Enzyme linked immunosorbent assay (ELISA) methods in different format.

Among these, ELISA has been reported as popular and commonly used immunological methods for the selection of STEC strain with high sensitivity and specificity. The detection mechanism is performed by capture antibody which is linked to an enzyme, or by a secondary enzyme-linked antibody targeting the capture antibody. Then, by adding the enzymatic substrate, a visible signal proportional to the amount of antigen is generated. There are different types of enzymes can be used in ELISA, some of the most commonly used enzymes that conjugate with secondary antibodies are horseradish peroxidase (HRP), alkaline phosphatase and beta-galactosidase [48]. Different ELISA format methods described in (Figure 5) such us; Direct ELISA (dELISA),Indirect ELISA (iELISA) , Competitive ELISA (cELISA) and Competitive ELISA (cELISA).

The disadvantage of immunodiagnostic assay kit is the chance of cross-reactivity which leads to false positive and decrease specificity of the assay (ELISA) [43]. Moreover, in addition to ELISA, very fast, specific, and efficient flow cytometer (comparison of genetic fragments) assay have been designed for the screening of the 6 major non-O157STECs [49]. And also a slide and

latex agglutination kit has been used for the detection of STEC serotype like O157, with fatly and easily than other methods. Slide agglutination test is the cheapest, simple, rapid and fast to perform the test, due to these characteristics slide agglutination based STEC detection is routine used in most clinical diagnostic laboratories (Figure 6) [50].

Figure 6: Slide agglutination test result image.

Target genes	Detection method
Stx1, Stx2, eae	SYBR Green RTm-PCR
fliC-H21	Taqman real-time-PCR
O157:H7 single nucleotides polymorphisms	SYBR Green real-time-PCR
Stx, eae, wzx-O26, wzx-O103, wzx-O111, wzx-O1121, wzx-O145, wzx-O157	Conventional multiplex PCR
Stx1, Stx2, uidAO157	SYBR Green RTm-PCR
O157, Stx	SYBR Green real-time-PCR
Sutilase, Stx1, Stx2	Conventional multiplex PCR
Stx1, Stx2	Taqman real-time-PCR
Stx1, Stx2, wzx-O103, wzy-O103	multiplex PCR
wzx-O26, eae-O103, wbdI-O111, ihp1 O145	Taqman real-time multiplex PCR
perO157, rfbE-O157, wzyO111, fliA-O26, fliC-O26	SYBR Green real-time-PCR
eae and tir variants, katP, espP, espD, ehxA, etpD, saa	Taqman real-time-PCR
Stx1, Stx2	FRET real-time-PCR
Stx1, eae	Taqman real-time multiplex PCR
Stx1, Stx2, eae O157:H7	Taqman real-time multiplex PCR
Stx2	Conventional PCR
rfbE, fliC, stx1, stx2, mobA, eae, hly, 16S rRNA	RT-PCR
Stx1, Stx2, eae, hly	FRET multiplex realtime-PCR
Stx1, Stx2, Stx2e	Conventional PCR
Stx1, Stx2, uidAO157	CM-PCR and Taqman RTm-PCR
fliC-H7, stx1, stx2, eaeO157	Conventional multiplex PCR
stx1, stx2, eae, hly, rfb-O111, rfb-O157	Conventional multiplex PCR
eaeO157, stx1, stx2, fliC-O157 and rfbEO157	Conventional multiplex PCR

Table 4: Polymerase chain reaction detection methods and target genes of STEC strain.

MOLECULAR TECHNIQUE FOR SHIGA TOXIN-PRODUCING *E. COLI* SCREENING

Besides phonotypical and immunological assays of STEC strain detection nucleic acid methods represent a rapid and robust alternative in the principle of detecting virulence factor genes of STEC. In recent years, many diagnostic laboratories have switched to polymerase chain reaction (PCR) as a diagnostic tool for screening and distinguish between stx1, stx2, and other putative virulence gene (eae, ehxA) in pathogenic STEC strain [51]. Polymerase chain reaction (PCR) is the most common methods for detection of virulent toxins and other virulence markers coding gene. Currently, there are a variety of molecular methods such as conventional

PCR, multiplex PCR, quantitative PCR (qPCR) as well as Real time PCR (RT-PCR) that applicable for STEC detection. Of these, multiplex PCR and real-time PCR are the most popular. Amplification of target genes of STEC strain DNA extracts from feces is less successful than from pure cultures, therefore to improve its sensitivity, careful preparation of the sample (DNA) is important [52]. Rough lysates or DNA extracts obtained from growth colonies, as well as mixed broth cultures, colony sweeps, or direct extracts of feces or foods used as DNA templates for PCR and its amplification products are detected by different staining (ethidium bromide) after separation by agarose gel electrophoresis. The occurrence of real time PCR makes it possible for simultaneous quantification and detection of *E. coli* [53].

A range of molecular techniques such as conventional PCR, RT-PCR, and PCR joined to mass spectrometry, and isothermal nucleic acid amplification, have been engaged in the identification of target gene associated with STEC strains [52]. Stx specific PCR assays are highly specific, sensitive and not time consumed. However, PCR detect the gene sequence that code stx toxin, but not the toxin itself and also it does not indicate whether (or by how much) a gene is actually expressed [54]. During molecular based STEC detection, several genes (Tab 4) related with virulence are frequently used such as rfbE encoding O-antigen of O157 STEC, flicC encoding H7, specific flagellar antigen, intimin encoding eaeA gene, hemolysin encoding hlyA gene and uidA (gusA) gene which encoding β-glucuronidase. To differentiate E coli in serotype PCR assay targets O-antigen gene clusters include the wzx (O antigen flippase) and wzy (O antigen polymerase) genes, because of their genetic variability among the different serogroups [55].

In general, molecular based detection of STEC strain is, sensitive and specific than the culture and immunoassays based methods. However, there are certain limitations including time taken include cell lysis and nucleic acid extraction, cross-contamination, unsuccessful reactions due inhibitory substance or competing DNA from the non-target cells, and also it does not able to distinguish the live and dead cells, due to that make it important to develop advanced methods [43].

OTHER EMERGING TECHNOLOGIES FOR STEC STRAIN DETECTION

Conventional pathogen bacteria screening methods, such as microbiological and biochemical are time-consuming and laborious. However, immunological or nucleic acid-based methods require extensive sample preparation and sophostification equipment and reagents [56]. Due to this, other improved detection methodology/technology have been developed, such as biosensors, DNA microarray, nanobiotechnology and others. Biosensors are the devices for pathogen identification which consist of three elements, which are a biological capture molecule (probes and antibodies), a method for converting capture molecule - target interactions into a signal and an output data [57]. The major advantage of the biosensors is that these can detect the pathogens at low detection limits with high specificity and sensitivity, but the biosensors will require highly specific and expensive instruments, with compatible computer software, to give accurate results. Hence, these methods may not be always cost-effective [56].

DEVELOPMENT OF IMMUNODIAGNOSTIC KIT FOR STEC DETECTION USING ANTIBODIES

Antigenic (immunogenic) parts of STEC selection, isolation and purification for antibody production

Antigen is any molecule that is recognized as non-self by immune system that able to induce humoral or cellular immune response. There are three most common features that antigen must have to be immunogenic such as: foreignness, high molecular weight (more than 6000 dalton) and chemical complexity. Cellular immune response against to any antigen is facilitated by T

lymphocytes and it is impossible to transfer between individual via serum transfusion. Whereas humoral immune response (targets extracellular antigens) are proteins and available in serum (antibodies) that it is possible to transferred between individual when serum is transfused [58]. In native form B-lymphocytes use membrane IgM (mIgM) to bind its specific antigen. Once mIgM and antigen molecules makes a complex which is then taken into the cell by receptor mediated endocytosis. The antigen digests into small peptides by endosome fuses with a lysosome. Endolysosome also fused with a vesicle which consist MHC II molecules that bounded peptide antigens. MHC II/antigen complex expressed on plasma membrane of B-lymphocyte. T cell receptor of a T helper lymphocyte then binds with MHC II/antigen and the T cell secretes cytokines that signals to B-lymphocyte to divide, proliferate differentiate and secrete antibodies [59].

STEC strains can be classified into serotypes by on their cell wall O antigen: part of lipopolysaccharide layer, in their flagella: H antigen and their Capsule: K antigen that can stimulate an immune response in animals. Due to the occurrence of different sugars and sugar linkages O antigen in LPS is an immune-dominant molecule [60] that is vital for the development of virulence and pathogenicity of bacterial species and it is the most specific and variable within serotype of STEC strain [61]. About 75% of the surface and 5% - 10% of the total dry weight of gram negative bacteria is LPS which is recognized by immune cells as a pathogen-associated molecule [62] that activates immune system vie triggering cytokine (TLR-4) [63]. Lipopolysaccharide (LPS) is a complex structure which comprises three parts as indicated in (Figure 7) such as lipid A, core oligosaccharide and O antigen. Lipid A is a highly preserved hydrophobic portion of LPS and contributes to the toxicity of the LPS [64]. Whereas “O” antigen is carbohydrate chain that is a polymer of immunogenic repeating oligosaccharides (1 unit - 40 units), which is variable between species and is a main contributor for serological specificity of STEC and the other component is core region which is phosphorylated nonrepeating oligosaccharide [65].

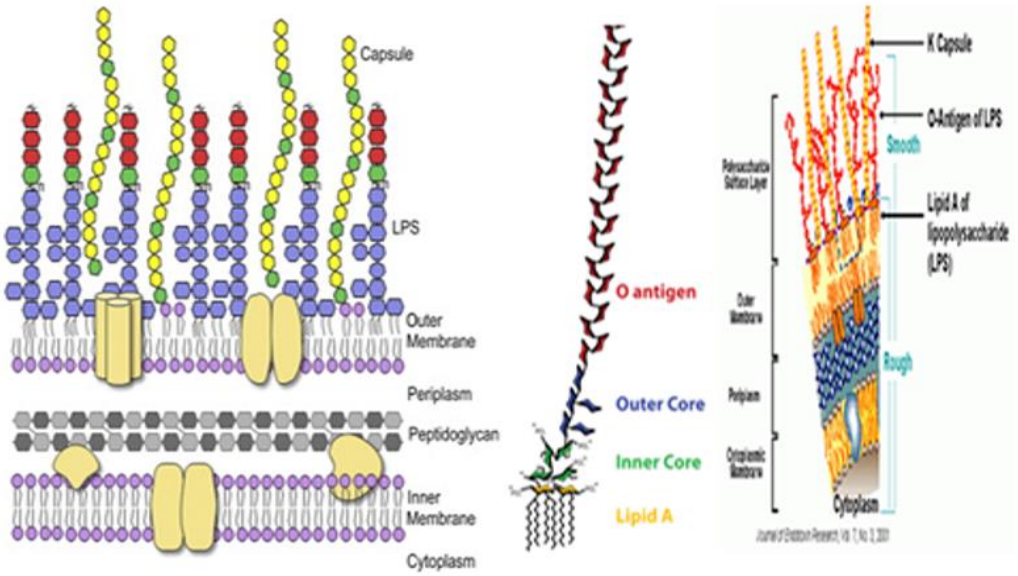

Figure 7: Structure of a lipopolysaccharide of STEC strain.

The genes that expressed O-antigens are located on the chromosome and classified into three main classes: (i) genes for biosynthesis of nucleotide sugars; (ii) genes for transfer of sugars from their respective nucleotide sugar donors to build O unit; (iii) genes for processing steps in the conversion of the O unit and carrying out specific assembly to form O-antigen in LPS, including flippase

gene (wzx) and polymerase gene (wzy) [62]. Currently, O5, O15, O26, O45, O55, O76, O91, O103, O104, O111, O113, O118, O121, O123, O128, O145, O146, O157, O165, O172, and O177 serotypes are recognized as clinically relevant O-antigen forms of Shiga toxin producing *E.coli* (STEC) [7]. Therefore, to design specific immunodiagnostic kit for STEC detection using antibody against STEC, isolation, purification and characterization of LPS is important step. However, contamination with capsular polysaccharide, nucleic acids and outer membrane proteins like peptidoglycan which potentially interfere with downstream process is the main problem with LPS purification procedures which hinder its reliable application in most downstream immunological and biological experiments [66]. Numerous methods have been developed for isolation and purification of LPS of STEC among which the Hot-Phenol method is a most frequently used with its limitation (cancerous, tedious and poisonous nature). However, methanol-chloroform method is easy, less expensive (economical), quick and safer with its drawback (contamination of LPS yielded with capsular polysaccharide, nucleic acids and outer membrane proteins, which are introduced to the final purified LPS during extraction and purification than Hot-Phenol method of LPS extraction. However, these contaminant proteins are removed by methanol-chloroform treatment because they are soluble in methanol-chloroform but LPS is not soluble [67].

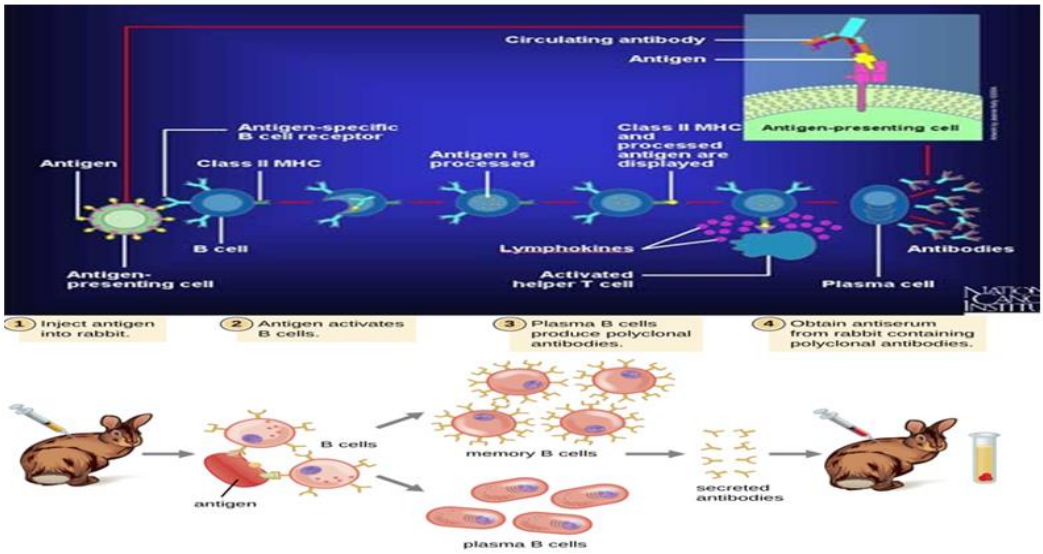

Figure 8: Antibody development (B-cell proliferate in to plasma and memory B-cell).

ANTIBODY DEVELOPMENT

To develop immunodiagnostic assay kit, antibodies production is important scheme either against a single antigen or antigens associated with a specific analyte, pathogen. For successful generation of antibodies depends on B-lymphocytes to bind, process and present antigen to T helper lymphocytes, that leads generate signals the specific B cells that have been activated by the antigen binding to the B cell's antigen-receptor to differentiate into antibody-producing cells (plasma cells) in the lymphoid organs (spleen, lymph nodes, among others) [68] as indicated in (Figure 8).

After primary immunization naive B cell are stimulated to multiply and differentiate in to plasma cells (fighting the current antigen) or memory cells (to address future antigen exposure). After the selected animals injected with the immunogenic, specific antibody begin to generate in the serum within 5 days to 7 days of immunized and their concentration is continuous to rise and peaks with 10 days to 14 days and after which it

decreases. Following the initial immunization, booster injections is important for a continue exposure of antigens to B cells development and the injected antigen is neutralized via a host humeral immune response (antibody), as the same time a small number of activated B cells will differentiate into memory cells, and most will die by apoptosis [69].

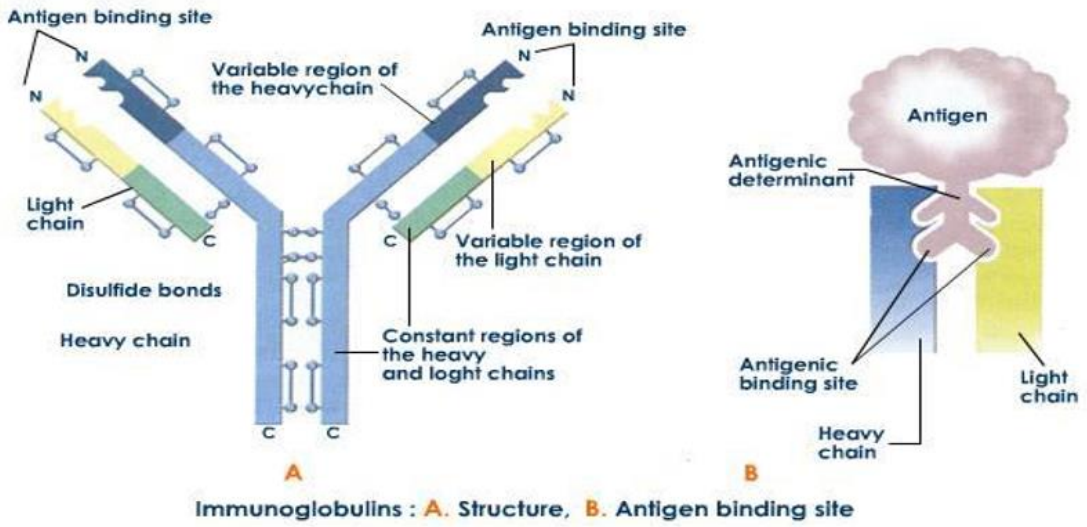

Figure 9: Structure of an antibody (immunoglobine) molecule.

However, the lag or adaptation period for specific antibody production is short and the peaks of antibody production (secondary antibody response stimulation) occurs 7 days to 14 days after boosting less amount of antigen immunized and their concentration sustained for a long period of [68]. Antibodies (or immunoglobulin) are a family of proteins of the adaptive immune system that defend the host by binding to antigens and they have a similar structure (constant region) in common that determines the functionality of the antibody and the variable region is responsible for binding the respective antigens. As showed in (Figure 9) antibody containing four polypeptide chains: two identical heavy chains (H) and two identical light chains (L) thus providing at least two sites capable of binding an antigen. Heavy chains are connected to each other by two and above disulfide bonds, whereas each light chain is connected to a heavy chain by one disulfide bond [70]. The amino (N) terminus of a light and heavy chain contains the hyper variable amino acid region, or the "Fab" portion of the antibody molecule, whereas the carboxylic acid (COOH) terminus of both heavy chains composes the crystallizable (Fc portion of the antibody). The V regions of H and L chains comprise the antigen-binding sites of the immunoglobulin molecules [71].

Antibodies are produced and purified as polyclonal and monoclonal form which used as reagents in immunoassays development. Antibodies those produced and secreted by a single clone of B lymphocytes are called monoclonal antibodies, and while those produced by a mixture of different cell lines of B lymphocytes clones (originate from common stem cells), termed as polyclonal antibodies which have heterogeneous immunological response to an antigen. Both antibodies are important tool in the area of immunological research, immunohistochemistry, diagnostic testing, and vaccine quality control [68].

A number of critical steps are developed for polyclonal and monoclonal antibodies production, such as preparation of antigen samples, selection of the animal species with their injection sit, selection and preparation

of the adjuvant, post-injection observation, and collection and processing of antibodies [72]. In the cause of polyclonal antibody production, adjuvant conjugate or antigen alone is injected into selected animal to initiate an amplified immune response. After a series of injections over a specific length of time, the animal is expected to have created antibodies against the conjugate. The polyclonal antibodies are acquired from a host via blood sampling, which involves collection from the host every 2 weeks and then purified by centrifuged the collected blood to obtain the antibody of interest [8].

Therefore, a polyclonal antiserum can be obtained within a short time (4 weeks - 8 weeks) with little financial investment. Whereas it takes about 3 to 6 month to produce MAbs. However, monoclonal antibodies are secreted from a single cell line through by fusing antibody-secreting spleen cells from immunized mice with immortal myeloma cell to create monoclonal hybridoma cell lines that express the specific antibody in cell culture supernatant [46]. The general polyclonal and monoclonal antibody production scheme and also immunoglobulin diversification and B cell development are described in (Figure 10 & Figure 11).

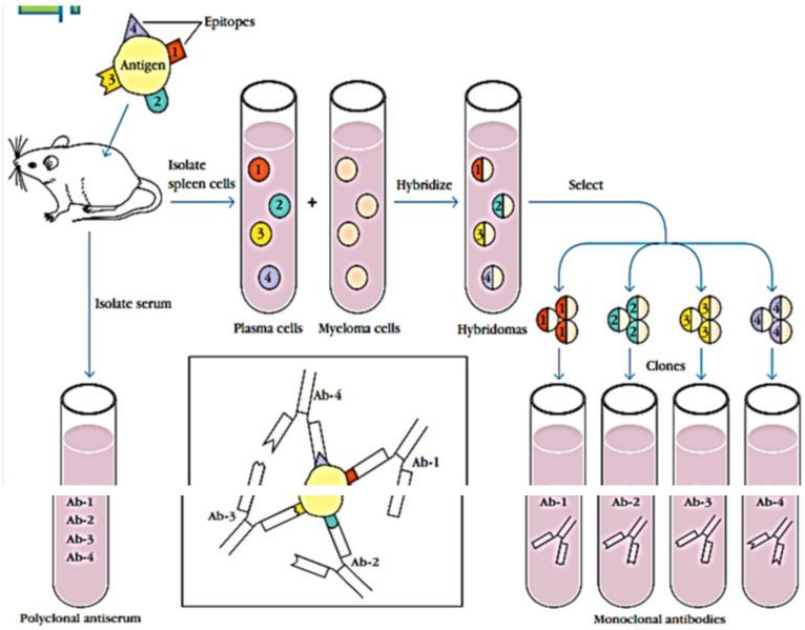

Figure 10: Polyclonal and monoclonal antibody production scheme.

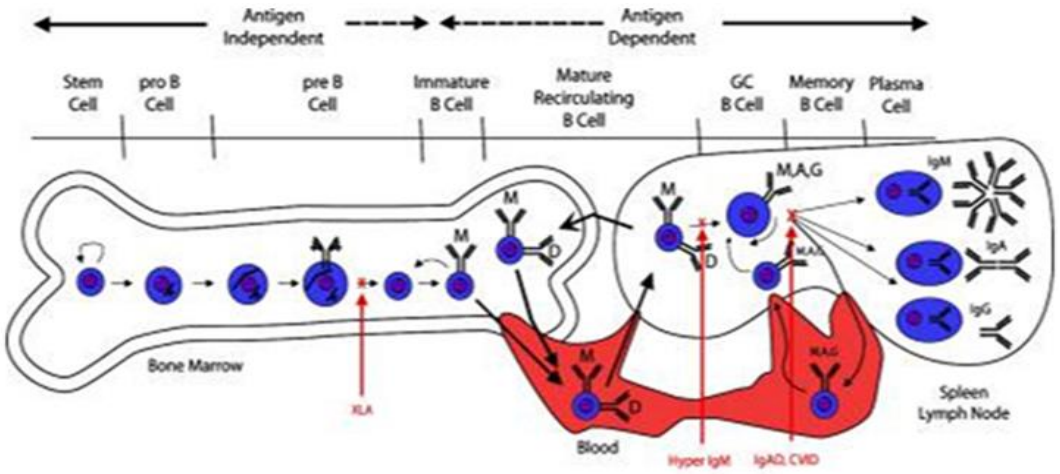

Figure 11: Immunoglobulin diversification and B cell development.

The first immunoglobulin class to be generated by B cells is IgM, which is the most efficient immunoglobine (900, 000 dalton MW) in agglutination reaction and a pentavalent with high affinity to antigen epitopes as primary immune response. IgM immune response declined with time and replaced by a second, high avidity immunoglobulin IgG (150, 000 dalton MW) antibodies as secondary immune response and others are generated with different concentration. The concentration of IgG, IgA, IgM, IgE and IgD in serum is approximately, 10 mg/mL - 16 mg/mL, 1 mg/mL - 4 mg/mL, 0.5 mg/mL - 2 mg/mL, 0.00001 mg/mL - 0.0004 mg/mL and 0 mg/mL - 0.4 mg/mL and their total concentration in serum in percentage is 80%, 10% - 15%, 5% - 10% , <0.002% and <0.2% respectively [73].

CONCLUSION

It is possible to conclude that Shiga toxin-producing *Escherichia coli* (STEC) are the leading pathogenic strain that causes severe human diseases. Even in some countries the some infectious disease will range from epidemic to pandemic that make the second and frequent cause of mortality globally. Through a year many screening methods to aid in the diagnosis of STEC strain have been developed; yet, many of the available diagnostic assays have different limitations such as time consuming, lack of sensitivity and specificity, require expensive equipment and trained personnel and also may not be readily available in many clinical areas. Henceforth; a new diagnostic should be designed so as to detect all STEC strains, other pathogenic *E. coli* and any disease causing microorganisms. Ultimately this review will have an immense role in the academia, universities and research institutions on how, and for what a new approach of kit designing is very crucial.

Perhaps the incidence of COVID -19 also play a trajectory and gear shifting on such kind of thought and will be a base for a new normal approach of test kit.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

REsFERENCES

1. Koev K, Zhelev G, Marutsov P, et al. (2018) Isolation and primary identification of shiga toxin producing *Escherichia coli* O157 in dairy cattle. Bulgarian Journal of Veterinary Medicine 21(4): 445-450.
2. James PN, James BK (1998) Diarrheagenic *Escherichia coli*. American Society for Microbiology 11: 142-201.
3. Donnenberg MS, Whittam TS (2001) Pathogenesis and evolution of virulence in enteropathogenic and enterohemorrhagic *Escherichia coli*. The Journal of Clinical Investigation 107 (5): 539-548.
4. Tania ATG, Waldir PE, Isabel CAS, et al. (2016) Diarrheagenic *Escherichia coli*. Brazile Journal of Microbiology 47: 3-30.
5. Clements A, Young JC, Constantinou N, et al. (2012) Infection strategies of enteric pathogenic *Escherichia coli*. Gut Microbes 3(2): 71-87.
6. James PN, Volker M, Judith J, et al. (2006) Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. Clinical Infectious Diseases 43(4): 402-407.
7. Sanchez S, Llorente MT, Echeita MA, et al. (2015) Development of three multiplex PCR assays targeting the 21 most clinically relevant serogroups associated with shiga toxin-producing *E. coli* infection in humans. PloS One 10(1): e0117660.
8. He X, Patfield S, Hnasko R, et al. (2013) A polyclonal antibody based immunoassay detects seven subtypes of Shiga toxin 2 produced by *Escherichia coli* in human and environmental samples. PloS One 8(10): e76368.

9. Wang J, Katani R, Li L, et al. (2016) Rapid detection of *Escherichia coli* O157 and shiga toxins by lateral flow immunoassays. Toxins 8(4): 92.
10. Geue L, Segura M, Conraths FJ, et al. (2002) A long-term study on the prevalence of shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. Epidemiology and Infection 129(1): 173-185.
11. Fratamico PM, DebRoy C, Liu Y, et al. (2016) Advances in molecular serotyping and subtyping of *Escherichia coli*. Frontiers in Microbiology 7: 644.
12. Athumani ML (2018) Epidemiology of shiga toxin-producing *Escherichia coli* O157:H7 in Africa in review. Journal of Infectious Diseases 33(1): 24-30.
13. Castro VS, Carvalho RC, Conte-Junior CA, et al. (2017) Shiga-toxin producing *Escherichia coli*: Pathogenicity, super shedding, diagnostic methods, occurrence, and foodborne outbreaks. Comprehensive Reviews in Food Science and Food Safety 16:1269-1280.
14. Majowicz SE, Scallan E, Jones-Bitton A, et al. (2014) Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: A systematic review and knowledge synthesis. Foodborne Pathogens and Disease 11(6): 447-55.
15. Botkin DJ, Galli L, Sankarapani V, et al. (2012) Development of a multiplex PCR assay for detection of shiga toxin-producing *Escherichia coli*, enterohemorrhagic *E. coli*, and enteropathogenic *E. coli* strains. Frontiers in Cellular and Infection Microbiology 2: 8.
16. Hajra TK, Bag PK, Das SC, et al. (2007) Development of a simple latex agglutination assay for detection of shiga toxin-producing *Escherichia coli* (STEC) by using polyclonal antibody against STEC. Clinical and Vaccine Immunology 14(5): 600-604.
17. Croxen MA, Law RJ, Scholz R, et al. (2013) Recent advances in understanding enteric pathogenic *Escherichia coli*. Clinical Microbiology Reviews 26(4): 822-880.
18. Norman KN, Strockbine NA, Bono, JL (2012) Association of nucleotide polymorphisms within the O-antigen gene cluster of *Escherichia coli* O26, O45, O103, O111, O121, and O145 with serogroups and genetic subtypes. Applied and Environmental Microbiology 78(18): 6689-6703.
19. Zhu C, Yu J, Yang Z, et al. (2007) Protection against Shiga toxin-producing *Escherichia coli* infection by transcutaneous immunization with Shiga toxin subunit B. Clinical and Vaccine Immunology 15: 359-366.
20. Ho NK, Henry AC, Johnson K, et al. (2013) Pathogenicity, host responses and implications for management of enterohemorrhagic *Escherichia coli* O157:H7 infection. Journal of Gastroenterology 27(5): 281-285.
21. Abdullah UH, Al-Sultan IA, Jassim HM, et al. (2014) Hemolytic uremic syndrome caused by shiga toxin producing *Escherichia coli* infections. Cloning & Transgenesis 3(2): 125.
22. Hoffman MA, Menge C, Casey TA, et al. (2006) Bovine immune response to shiga-toxigenic *Escherichia coli* O157:H7. Clinical and Vaccine Immunology 13(12): 1322-1327.
23. Branger J, Knapp S, Weijer S, et al. (2004) Role of Toll-like receptor 4 in gram-positive and gram-negative pneumonia in mice. Infection and immunity 72(2): 788-794.
24. Judith H, Emily M, Zanzo Z, et al. (1997) Antiserum against *Escherichia coli* J5 contains antibodies reactive with outer membrane proteins of heterologous Gram-Negative bacteria. The Journal of Infectious Diseases 176(5): 1260-1268.
25. Iwasaki A, Medzhitov R (2010) Regulation of adaptive immunity by the innate immune system. Science 327: 291-295.
26. Roger T, Froidevaux C, Le Roy D, et al. (2009) Protection from lethal gram-negative bacterial sepsis by targeting Toll-like receptor 4. Proceedings of the National Academy of Sciences of the United States of America 106(7): 2348-2352.
27. Nasim D, Shaghayegh J, Vaseghi G (2017) Effect of lipopolysaccharide on toll-like receptor-4 signals in mouse cancer cells. Bratisl Medical Journal 118(10): 598-601.

28. Proulx F, Seidman GE, Karpman D (2001) Pathogenesis of shiga toxin associated hemolytic uremic syndrome. Pediatric Research 50: 163-171.
29. Mak TW, Saunders ME (2006) Perspective on immunity and immunology. The Immune Response: 3-16.
30. Sang WK, Boga H, Waiyaki PG, et al. (2012) Prevalence and genetic characteristics of shiga toxigenic *Escherichia coli* from patients with diarrhoea in Maasail and, Kenya. Journal of Infection in Developing Countries 6(2): 102-108.
31. Monaghan A, Byrne B, Fanning S, et al. (2011) Serotypes and virulence profiles of non-O157 shiga toxin-producing *Escherichia coli* isolates from bovine farms. Applied and Environmental Microbiology **77** (24): 8662-8668.
32. Hannah G, Cheryl B, Nancy S, et al. (2009) Recommendations for diagnosis of shiga toxin- producing *Escherichia coli* infections by clinical laboratories. American Society for Microbiology 58(12): 1-4.
33. Skinner C, Patfield S, Stanker L, et al. (2013) Development of monoclonal antibodies and immunoassays for sensitive and specific detection of shiga toxin Stx2f. PLoS One 8(9): e76563.
34. Vallieres E, Jean MS, Rallu F (2013) Comparison of three different methods for detection of shiga toxin Producing *Escherichia coli* in a tertiary pediatric care Center. Journal of Clinical Microbiology 51: 481-486.
35. William JZ, Max T, Clifton KF (2016) Shiga toxin producing *Escherichia coli*: detection, differentiation, and implications for food safety. IFAS Extension.
36. Priyanka B, Patil RK, Dwarakanath S (2017) A review on detection methods used for foodborne pathogens. The Indian Journal of Medical Research 144(3): 327-338.
37. March SB, Ratnam S (1986) Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. Journal of Clinical Microbiology 23(5): 869-872.
38. Linda C, Marc RC, Theodore C, et al. (2010) Comparison of shiga toxin producing *Escherichia coli* detection methods using clinical stool samples. The Journal of Molecular Diagnostics 12(4): 469-475.
39. Sata S, Fujisawa T, Osawa R, et al. (2003) An improved enrichment broth for isolation of *Escherichia coli* O157, with specific reference to starved cells, from radish sprouts. Applied and Environmental Microbiology 69(3): 1858-1860.
40. Gouali M, Ruckly C, Carle I, et al. (2013) Evaluation of CHROMagar STEC and STEC O104 chromogenic agar media for detection of shiga toxin-producing *Escherichia coli* in stool specimens. Journal of Clinical Microbiology 51(3): 894-900.
41. Feng P, Weagan SD, Jinneman K (2017) Bacteriological analytical manual: Diarrheagenic *Escherichia coli.* US Food and Drug Administration, USA.
42. Verhaegen B, Damme IV, Heyndrickx M, et al. (2016) Evaluation of detection methods for non-O157 Shiga toxin-producing *Escherichia coli* from food. International Journal of Food Microbiology 219: 64-70.
43. Parsons BD, Zelyas N, Berenger BM, et al. (2016) Detection, characterization, and typing of shiga toxin producing *Escherichia coli.* Frontiers in Microbiology 7: 478.
44. Li W, Zhou J, Xu Y (2015) Study of the *in vitro* cytotoxicity testing of medical devices. Biomedical Reports 3(5): 617-620.
45. Gerritzen A, Wittke JW, Wolff D (2011) Rapid and sensitive detection of shiga toxin producing *Escherichia Coli* directly from stool samples by real-time PCR in comparison to culture, enzyme immunoassay and vero cell cytotoxicity assay. Clinical Laboratory 57: 993-998.
46. Peruski AH, Peruski LF (2003) Immunological methods for detection and identification of infectious disease and biological warfare agents. Clinical and Diagnostic Laboratory Immunology 10(4): 506-513.
47. Darwish IA (2006) Immunoassay methods and their applications in pharmaceutical analysis: Basic methodology and recent advances. International Journal of Biomedical Science 2(30): 217-230.
48. Law JW, AbMutalib NS, Chan KG, et al. (2014) Rapid methods for the detection of foodborne bacterial pathogens: Principles, applications, advantages and limitations. Frontiers in Microbiology 5: 770.

49. Hegde NV, Jayarao BM, DebRoy C (2012) Rapid detection of the top six non-O157 shiga toxin-producing *Escherichia coli* O groups in ground beef by flow cytometer. Journal of Clinical Microbiology 50(6): 2137-2139.
50. Hatch WO, Scalarone GM (2013) Development of a slide agglutination assay for detection of blastomycosis. Microbiology and Immunology 57:756-761.
51. Haugum K, Brandal LT, Lindstedt BA, et al. (2014) PCR-based detection and molecular characterization of shiga toxin-producing *Escherichia coli* strains in a routine microbiology laboratory over 16 years. Journal of Clinical Microbiology 52(9): 156-163.
52. Quiros P, Martínez-Castillo A, Muniesa M (2015) Improving detection of Shiga toxin-producing *Escherichia coli* by molecular methods by reducing the interference of free shiga toxin-encoding bacteriophages. Applied Environmental Microbiology 81: 415-421.
53. Asmelash T (2015) Isolation, identification, antimicrobial profile and molecular characterization of Enterohaemorrhagic *E. coli* O157:H7 isolated from ruminants slaughtered at Debre Zeit ELFORA export abattoir and Addis Ababa Abattoirs Enterprise 1-79.
54. Fagerquist CK, Zaragoza WJ (2015) Shiga toxin 2 subtypes of Enterohemorrhagic *E. coli* O157: H- E32511 analyzed by RT-qPCR and top-down proteomics using MALDI-TOF-TOF-MS. Journal of American Society for Mass Spectrometry 26: 788-779.
55. Liu Y, Yan X, DebRoy C, et al. (2015) *Escherichia coli* O-Antigen gene clusters of serogroups O62, O68, O131, O140, O142, and O163: DNA sequences and similarity between O62 and O68, and PCR-Based serogrouping. Biosensors 5(1): 51-68.
56. Ahmed A, Rushworth JV, Hirst NA, et al. (2014) Biosensors for whole-cell bacterial detection. Clinical Microbiology 27 (3): 631-646.
57. Chen S, Cheng YF (2017) Biosensors for bacterial detection. International Journal of Biosensors & Bioelectronics 2(6): 197-199.
58. Osato K (1972) Antigen-antibody complexes in the immune response, analysis of the effectiveness of complexes on the primary antibody response. Immunology 23(4): 545-57.
59. Wieczorek M, Abualrous ET, Sticht J, et al. (2017) Major histocompatibility complex (MHC) Class I and MHC Class II Proteins: Conformational plasticity in antigen presentation. Frontiers in Immunology 8: 292.
60. Senchenkova SN (2006) Structural and genetic characterization of *Shigella boydii* type 17 O 69 antigen and confirmation of two new genes involved in the synthesis of glucolactilic acid. Biochemical and Biophysical Research Communications 349: 289-295.
61. Zhang G, Meredith TC, Kahne D (2013) On the essentiality of lipopolysaccharide to Gram-negative bacteria. Current Opinion in Microbiology 16(6): 779-785.
62. Beutin L, Wang Q, Naumann D, et al. (2007) Relationship between O-antigen subtypes, bacterial surface structures and O-antigen gene clusters in *Escherichia coli* O123 strains carrying genes for Shiga toxins and intimin. Journal of Medical Microbiology 56:177-184.
63. Sohrabi S, Akbarzadeh A, Norouzian D, et al. (2011) Production and purification of rabbit's polyclonal antibody against factor VIII. Indian Journal of Clinical Biochemistry 26(4):354- 359.
64. Rezania S, Amirmozaffari N, Tabarraei B, et al. (2011) Extraction, purification and characterization of lipopolysaccharide from *Escherichia coli* and *Salmonella typhi*. Avicenna Journal of Medical Biotechnology 3(1): 3-12.
65. Micoli F, Rondini S, Gavini M, et al. (2013) A scalable method for O-antigen purification applied to various Salmonella serovars. Analytical Biochemistry 33: 136-145.

66. Carol G, Currie I, Poxton R (1999) The lipopolysaccharide core type of *Escherichia coli* O157:H7 and other non-O157 verotoxin-producing *E. coli.* FEMS Immunology and Medical Microbiology 24: 57-62.
67. Kalambhe DG, Zade NN, Chaudhari SP (2017) Evaluation of two different lipopolysaccharide extraction methods for purity and functionality of LPS. International Journal of Current Microbiology and Applied Sciences 6(3): 1296-1302.
68. Leenaars M, Hendriksen CF (2005) Critical steps in the production of polyclonal and monoclonal antibodies: Evaluation and recommendations. Institute for Laboratory Animal Research Journal 46(3): 269-279.
69. Pappas MG (1994) Immunodiagnostic assays. In: The biotech business handbook. Humana Press, Totowa, NJ, USA: 247-248.
70. Janeway CAJ, Travers P, Walport M (2001) Immunobiology. In: The immune system in health and disease, 5th (Edn.), New York: Garland Science; The structure of a typical antibody molecule.
71. Kirkham PM, Schroeder HW (1994) Antibody structure and the evolution of immunoglobulin V gene segments. Seminars in Immunology 6: 347-360.
72. Boer E, Heuvelink A (2000) Methods for the detection and isolation of shiga toxin-producing *Escherichia coli*. Journal of Applied Microbiology 88: 133-143.
73. Schroeder HW, Cavacini L (2010) Structure and function of immunoglobulins. The Journal of Allergy and Clinical Immunology 125: 41-52.