DETECTION OF ROTAVIRUS IN DIARRHEAL PATIENTS

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May, 2016

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Submitted in the partial fulfillment of the degree of

Bachelor of Technology

ABSTRACT

Around 800,000 children die every year from diarrhoea globally. In India alone around 300,000 deaths occur. Viral infection is the leading cause of diarrhoea and even in this category *Rotavirus* accounts for majority of cases. In India rotavirus associated diarrheal cases accounts for 100,000 deaths and so its detection and monitoring is quite necessary. Rotaviruses are classified into 7 groups (A-G) on the basis of amino acid sequence of their VP6 protein. Another traditional method used for classification of rotaviruses is G/P genotyping – defining the two neutralizing antigens on the outer capsid – VP4 (a protease sensitive protein protruding from the surface and labeled as the P-type) and VP7 (an outer capsid glycoprotein labeled as the G-type). Human rotaviruses constitute a diverse group. Until now, 27 G genotypes (G1–G27) and 35 P genotypes (P[1] – P[35]) have been detected. Most commonly isolated G and P types are G1, G2, G3, G4, G9 and P[4], P[8] respectively. ELISA and RT-PCR are the most common diagnostic techniques and have been used in this study. The specificity of ELISA and sensitivity of RT-PCR have been targeted for detection of rotavirus in fecal samples. Of all analysed samples, 22.9% are rotavirus positive and its dominance in children (under the age group of 5 years) is more as compared to adults. These techniques can be elaborately used for large studies and thus, help in establishing epidemiological data for prevalent rotavirus strains that can further help in developing effective vaccination strategies.

ACKNOWLEDGEMENTS

I am truly grateful to **Dr. Harish Changotra**, Associate professor, Department of Biotechnology & Bio informatics, Jaypee University of Information & Technology (JUIT), for accepting me in his lab and introducing me to this research topic. His calm and friendly nature always made me feel comfortable. The passion he has for his work has motivated me to pursue my goals more diligently.

I am thankful to Dr. R. S. Chauhan, HOD, Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology for his encouragement and for providing me with this opportunity and facilities to carry out the project work and guiding me whenever required. I also acknowledge the guidance and helpful comments of all the professors of the department of Biotechnology & Bioinformatics.

I sincerely thank my mentor **Mr. Swapnil Jain** who has been a biggest source of encouragement. I am especially grateful to him for showing utmost level of patience while I was in my initial learning stage and he gracefully helped me to overcome my deficiencies. His positive attitude always motivated me to move ahead. This work would not have been possible without him.

I am grateful to all the other PhD scholars –Mrs Shivani, Ms Poonam, and Ms Nutan who have patiently extended all sorts of help for accomplishing this undertaking. I would also like to thank Baleshwar ji and Mrs Sonica for their constant support in the lab. I am thankful to all the wonderful people who helped me either through their sound suggestion or letting me use their lab equipments for the completion of many of the experiments.

At last but not the least, I am heartily thankful to my parents whose support and love helped me get through some of the toughest moments and always encouraged me to perform better and never give up the hope.

DECLARATION

I hereby declare that the work reported in the B-Tech thesis entitled "**Detection of Rotavirus in Diarrheal Patients**" submitted at Jaypee University of Information Technology, Waknaghat India, is an authentic record of my work carried out under the supervision of Dr. Harish Changotra. I have not submitted this work elsewhere for any other degree or diploma.

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CERTIFICATE

This is to certify that the work titled "**Detection of Rotavirus in Diarrheal Patients**", submitted by Sumant Anurag (121556) in partial fulfillment for the award of 4 year degree program, Bachelors of Technology at Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or fully to any other university or institute for the award of this or any other degree or diploma.

Supervisor:
Dr. Harish Changotra
Associate Professor (Biotechnology)
Date-

LIST OF ACRONYMS

nt	NUCLEOTIDE
TBM	TETRAMETHYLBENZIDINE
EM	ELECTRON MICROSCOPY
VP	VIRAL PROTEIN
NSP	NON STRUCTURAL PROTEIN
ELISA	ENZYME LINKED IMMUNOSORBENT ASSAY
RT-PCR	REVERSE TRANSCRIPTASE - POLYMERASE CHAIN REACTION
PBS	PHOSPHATE BUFFERED SALINE
RPM	ROTATION PER MINUTE
DMSO	DIMETHYL SULFOXIDE
TAE	TRIS-ACETATE-EDTA

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CHAPTER 1

Introduction

Diarrhea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Frequent passing of formed stools is not diarrhea, nor is the passing of loose, "pasty" stools by breastfed babies. Around 800,000 children die every year from diarrhoea globally. In India alone around 300,000 deaths occur [1] and it has been the second most common cause of death in the category of infectious diseases after pneumonia (Figure 1). There are three categories of causative agents: (1) viral, examples, *Rotavirus*, *Norovirus* and *Adenovirus*; (2) bacterial, examples, *Campylobacter*, *Salmonellae*, and *Shigella* and (3) parasitic, examples, *Giardia lambia* and *Cryptosporidium*. Amongst these, viral infection is the leading cause of diarrhoea and even in this category *Rotavirus* accounts for majority of cases. Rotavirus is the leading cause of diarrhea in children and is responsible for around 500,000 deaths globally [2] (mainly in developing countries). Of these 500,000 deaths, India bears the highest burden of rotavirus associated deaths with around 100,000 deaths every year, i.e. twenty percent of all rotavirus deaths under five years of age happened in India. Nigeria, the Democratic Republic of the Congo, Pakistan, India, and Ethiopia together accounts for almost half of all the rotavirus associated deaths under age five.

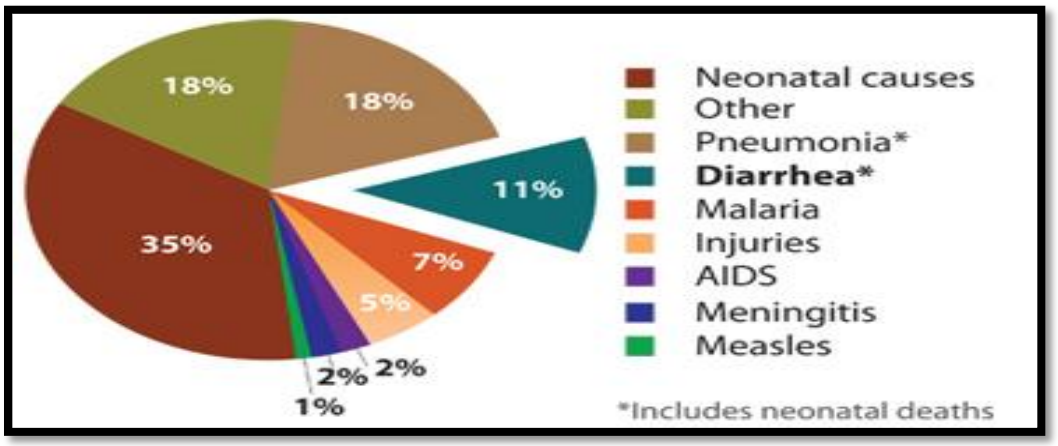


Figure 1: Pie chart depicting leading causes of death among children [3].

Rotaviruses (*rota* in Latin means wheel) have a wheel-like appearance and were first observed by Bishop and colleagues in 1973 [4]. They belong to the family Reoviridae and have a genome of 11 segments of double-stranded RNA enclosed within a triple layered capsid (Figure 2). Each genome segment encodes at least one protein – 6 structural (VP1-VP4, VP6, and VP7) and 6 non-structural (NSP1-NSP6). The genome size is approximately 18,550 bp and the RNA segments have length varying from 667 to 3302 nucleotides. VP4 and VP7 proteins form the outermost shell of the virus; the interm ediate layer is composed of VP6 protein; and VP1, VP2 and VP3 proteins assemble to form the inner most layer. Rotaviruses are classified into 7 groups (A-G) on the basis of amino acid sequence of their VP6 protein [5]. Another traditional method used for classification of rotaviruses is G/P genotyping – defining the two neutralizing antigens on the outer capsid – VP4 (a protease sensitive protein protruding from the surface and labeled as the P-type) and VP7 (an outer capsid glycoprotein labeled as the G-type) [6]. Human rotaviruses constitute a diverse group. Until now, 27 G genotypes (G1–G27) and 35 P genotypes (P[1] – P[35]) have been detected [7]. Most commonly isolated G and P types are G1, G2, G3, G4, G9 and P[4], P[8] respectively. The genes encoding VP7 and VP4 proteins segregate independently and give rise to a large number of G-P combinations. Studies reveal the existence of more than 70 different G-P combinations. Out of these, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] are most commonly identified G-P combinations and accounts for around 74% of rotavirus infections globally.

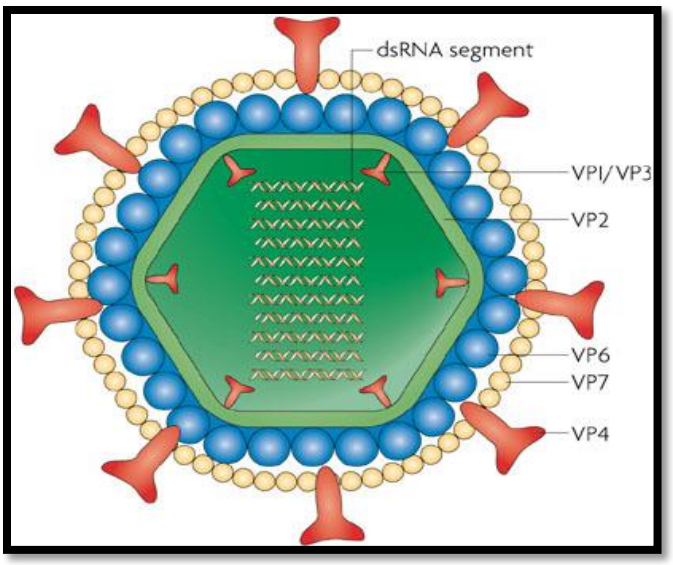


Figure 2: Schematic representation of Rotavirus structure.

The rationale for this study is to learn the basic and most commonly used techniques for detection of rotavirus associated diarrheal cases. ELISA and RT-PCR have been used in this study for the same. There are several advantages of identification and consequently genotyping the rotavirus strains. It'll help in knowing the prevalent strains in Himachal Pradesh and thus, will help in understanding the demographic distribution of rotavirus. This will help in developing

effective vaccination programs (as there has been no recent study done to monitor the circulating rotavirus strains in Himachal Pradesh). Thus, the total burden of rotavirus associated diarrheal deaths can be controlled with proper monitoring and surveillance.

CHAPTER 2

Review of Literature

At its core, diarrhea is simply an altered movement of ions and water that follows an osmotic gradient. Under normal conditions, the gastro-intestinal tract has tremendous capacity to absorb fluid and electrolytes, where 8–9 liters of fluid are presented to the intestine daily and only 100–200 ml are excreted in the stool. Enteric pathogens, however, can alter this balance towards net secretion, leading to diarrheal disease [8]. Diarrhea is a leading cause of morbidity and mortality across all age groups and regions of the world. Among children 0-59 months of age, diarrhea is responsible for 1.236 million deaths annually and is the second leading cause of death in this age group. Though mortality rates among older children, adolescents, and adults are lower than those observed in children under five, diarrhea still poses a substantial burden accounting for approximately 2.8 billion diarrhea episodes among older children, adolescents, and adults [9]. Diarrhea is associated with the three classes of infectious agents, i.e., bacteria (*Vibrio cholera, Clostridium difficile* and Shigella species), viruses (rotavirus, norovirus and astrovirus) and parasites (*Giardia lamblia* and *Entamoeba histolytica).* It is most commonly due to viral infection [10].

2.1 Structure

A rotavirus is a wheel-formed infection that gets its name from its intricate shape ('rota' implies wheel in Latin). Its genome comprises of 11 twofold stranded RNA sections that produce six viral proteins (VP1, VP2, VP3, VP4, VP6 and VP7) and six non-structural proteins (NSP1-6) (Table 1). Every infection molecule as demonstrated is encompassed by a triple layer coat made out of the diverse viral proteins. The external protein coat is made of VP4 and VP7 proteins. The VP4 shapes spikes on the external coat and appends the virion to the host cell, assuming a key part in cell infiltration and harmfulness. Rotavirus' VP7 protein, which frames the vast majority of the external layer, is the principle focus for the host's defensive antibodies. VP6 shapes the

center shell, or capsid, of the virion and is the most antigenic of the rotavirus basic proteins. The internal center of a rotavirus comprises of RNA strands and three basic proteins. VP2 is the principle basic part of the deepest layer. Alongside the viral RNA, two extra structural proteins, VP1 and VP3, are encased inside the VP2 layer and assume a critical part in RNA replication.

Table 1: Rotavirus gene segments and the proteins coded by them [11].

Protein	**Gene segment**	**Size of the protein (kD)**
VP1	1	125,00
VP2	2	94,000
VP3	3	88,000
VP4	4	88,000
NSP1	5	53,000
VP6	6	41,000
NSP3	7	34,000
NSP2	8	35,000
VP7	9	38,000
NSP4	10	28,000
NSP5	11	26,000
NSP6	11	12,000

2.1.1 Genome Structure

The viral genome of 11 sections of dsRNA is contained inside the infection center capsid. The sections range in size from 667 (fragment 11) to 3,302 base sets (portion 1), with the aggregate genome containing roughly 18,522 base sets (Table 1). This number, ordered from arrangement information of sections from various infection strains, concurs intimately with the genome size (18,680 base sets) dictated by EM estimations [12]. Hydrodynamic investigations of the adaptability or firmness of separated rotavirus RNA portions in arrangement have shown that

these RNA sections can't be bundled into the rotavirus capsid unless personal protein-RNA collaborations happen. In arrangement, these RNA particles have a "wormlike" or adaptable barrel structure; as an illustration, RNA portion 1 (3,302 base sets and a form length of 928 nm) hypothetically can't be twisted into a capsid of 50 nm as a free atom on the grounds that the constancy length is 112.5 nm [13]. In this way, to acquire RNA adaptability, one needs to expect that close protein-RNA associations happen in the virion to prompt the required bowing and bundling of the dsRNA fragments into the infection capsid. The proteins straightforwardly in charge of section bundling stay misty. The auxiliary proteins present in center particles (VP1, VP2, and VP3) are evident hopefuls; however non-structural proteins may likewise assume a framework part. Deproteinized, cleaned rotavirus dsRNAs are not irresistible, mirroring the way that infection particles contain their own RNA-subordinate RNA polymerase required to translate the individual RNA fragments into dynamic delivery person RNAs (mRNAs) [14].

2.2 Pathophysiology of Rotavirus Diarrhea

The enterocytes covering the small digestive tract are for the most part separated into two sorts: enterocytes and grave cells [15]. Villus enterocytes are full grown, nonproliferating cells covering the villi that are separated to digestive and absorptive capacities. The absorptive enterocytes orchestrate various disaccharidases, peptidases, and different chemicals that are communicated on the apical surface, where they do their digestive capacities. Retention over the enterocyte hindrance happens both by uninvolved dissemination of solutes along electrochemical or osmotic slopes and by dynamic transport. While the dominant part of water transport is aloof along osmotic inclinations, transporters, for example, the sodium-glucose co-transporter 1 (SGLT1) transport water alongside solute [15]. The tomb epithelium lines the graves and is the forebear of the villus enterocytes. Tomb cells do not have the very much characterized microvilli and absorptive elements of the enterocyte and effectively emit Cl^- particles into the intestinal lumen. In the typical creature, the joined movement of the enterocytes and sepulcher cells results in a consistent bidirectional flux of electrolytes and water over the epithelium. On the villi, the equalization is toward retention, and in the tombs, the parity favours emission.

Rotaviruses reproduce in the non-dividing adult enterocytes close to the tips of the villi, recommending that separated enterocytes express variables required for productive disease and replication [16]. The seriousness and confinement of rotavirus intestinal disease shift among creature species and between concentrates; in any case, the obsessive changes are only restricted to the small digestive tract. In different creature models, rotavirus contamination is connected with practically no noticeable sores; slight sores, for example, enterocyte vacuolization and misfortune; or bigger changes, for example, villus blunting and sepulcher hyperplasia. Aggravation is by and large mellow contrasted with that for other intestinal pathogens. This photo of pathology proposes that there is no supreme connection between's histological injuries and infection indications.

Rotavirus contamination changes the capacity of the little intestinal epithelium, bringing about looseness of the bowels. The loose bowels was by and large thought to be malabsorptive, optional to enterocyte demolition [17]. Notwithstanding enterocyte annihilation, assimilation of Na+, water, and mucosal disaccharidases are diminished [18], while mucosal cyclic AMP shows up not to be changed [19]. Malabsorption results in the travel of undigested mono-and disaccharides, sugars, fats, and proteins into the colon. The undigested bolus is osmotically dynamic, and the colon can't retain adequate water, prompting an osmotic looseness of the bowels. Another study recommended that the loose bowels was malabsorptive and came about because of epithelial harm brought on by villus ischemia [20]. A secretory segment of the looseness of the bowels was recommended, in view of lifted levels of prostaglandin E2 (PGE2) in the tainted gut and the incitement of emission by PGE2 [21]. The way that gut sores frequently don't relate with the nearness of loose bowels fortified the quest for different components of looseness of the bowels actuation. The viral non-structural protein NSP4, an emitted part of NSP4, or certain NSP4 peptides were found to have poison like movement and to actuate the runs when immunized into mice [22]. The NSP4 enterotoxin action gives an approach to intercede diarrheagenic changes without huge harm or to intervene changes at uninfected destinations. As of late, it was demonstrated that few medications that piece the activity of the ENS lessen rotavirus-impelled discharge in the digestive tract, recommending a part for the ENS in rotavirus the runs [23]. It was evaluated that ~67% of the liquid and electrolyte emission in rotavirus looseness of the bowels in trials with mice was because of initiation of the ENS [23].

2.2.1 Intestinal Infection

Rotavirus contamination can bring about asymptomatic or symptomatic disease. The result of disease is influenced by both viral and host elements. The most conspicuous host calculate that influences the clinical result of disease is age. In this manner, neonates contaminated with rotavirus once in a while have symptomatic illness; this security is thought to be intervened essentially by transplacental exchange of maternal antibodies [24]. Decreases in these antibodies harmonize with the time of most extreme weakness of newborn children to serious rotavirus-incited malady (3 months to 2 years). Rotavirus can contaminate grown-ups; however serious symptomatic ailment is generally exceptional and can come about because of diseases with a bizarre infection strain or to great degree high dosages of infection.

Infection destructiveness is identified with properties of the proteins encoded by a subset of the 11 viral genes. Infection destructiveness is multigenic and has been connected with genes 3, 4, 5, 9, and 10. The premise for the contribution of these qualities is just mostly caught on. Gene 3 encodes the topping catalyst that influences levels of viral RNA replication; genes 4 and 9 create the external capsid proteins required to start contamination. Gene 5 codes for a protein (NSP1) those capacities as an interferon rival (examined underneath in the insusceptibility segment). Gene 10 codes for the non-structural protein NSP4, which capacities to control calcium homeostasis, infection replication and as an enterotoxin.

Looseness of the bowels is the principle clinical appearance of rotavirus disease in newborn children and youthful kids. A sign of viral-incited looseness of the bowels that recognizes it from bacterial-actuated the runs is that little irritation is seen in contaminated entrails. Rotavirus principally contaminates intestinal villus enterocytes and grave cells are saved. Our comprehension of malady pathogenesis is constructing principally in light of studies in an assortment of creature models. Ailment pathogenesis is multifactorial and malabsorptive the runs happen because of infection interceded obliteration of absorptive enterocytes, infection prompted down-regulation of the outflow of absorptive chemicals, and useful changes in tight intersections between enterocytes that prompt paracellular spillage. There is a secretory part of rotavirus the runs that is thought to be intervened by enactment of the enteric sensory system and the impacts of NSP4—the initially depicted infection encoded enterotoxin . Investigations of the infection and the impacts of NSP4 alone, in refined cells and creature models, show that rotavirus-

instigated loose bowels results, to some degree, from actuation of cell Cl− channels, which expands emission of Cl− and thusly water. This Cl− discharge does not happen through the cystic fibrosis transmembrane controller—rotavirus and NSP4 prompt looseness of the bowels in mouse pups that do not have this direct and additionally in youngsters with cystic fibrosis [25]. Villus ischemia and modifications in intestinal motility have additionally been accounted for in some creature models however their part in illness in youngsters remains inadequately recorded.

2.2.2 Systemic Infection

Rotavirus contamination is not restricted to the digestive tract—its additional intestinal spread was archived more than 45 years back in mice, when infection was recognized in numerous organs [26]. These studies were to a great extent overlooked until touchy procedures re-assessed systemic contamination in an assortment of creature models and in children [27]. Obviously all tainted people and creatures experience no less than a brief time of viremia and infection can be identified in the few different tissues of immunocompetent hosts. The clinical outcomes of such systemic disease stay vague. In spite of the fact that there are numerous case reports partner rotavirus with numerous systemic ailments, there is no confirmation of causation from extraintestinal spread of rotavirus; this would be hard to demonstrate on the off chance that this type of the malady is uncommon. Be that as it may, it is critical for clinicians to think about how possible it is of systemic diseases and to be sensitive to conceivable cases in which causation can be appeared. It is not known whether the most as of late grew, live lessened antibodies result in viremia, yet unforeseen systemic diseases have not been.

2.3 The Molecular Basis of Diarrhea Induction

Rotavirus is the main source of life-undermining diarrheal illnesses among youthful kids. Research in the course of recent years has given essential bits of knowledge into instrument of viral pathogenesis and prompted effective improvement of live, constricted antibodies for gastroenteritis [28]. Rotavirus fundamentally taints little intestinal villus cells and can bring

about watery the runs with no huge intestinal aggravation. The double stranded RNA genome of rotavirus encodes for 6 structural proteins that shape infection particles (VPs) and 6 non-structural proteins (NSPs). NSP4 is the initially portrayed infection encoded enterotoxin and has been recommended to assume a basic part in liquid and electrolyte discharge [29]. Curiously, NSP4 is truant in the full grown infective virion and is orchestrated in tainted villus enterocytes. NSP4 and infection particles are discharged through the apical layer of energized epithelial cells by a non-traditional secretory pathway. In any case, NSP4 is additionally discharged from the basolateral side of tainted enterocytes, despite the fact that the part of basolaterally-discharged NSP4 in looseness of the bowels is not unmistakably comprehended. The virology and pathogenesis of rotavirus has been widely audited as of late. As opposed to established secretory the runs, the viral enterotoxin, NSP4, prompts looseness of the bowels consequent to maldigestion of starches attending with diminished water assimilation, expanded Ca2+ activation and a moderately gentle Cl− secretory segment. Maldigestion of sugars has been recommended as a noteworthy component hidden the pathophysiology of rotavirus-instigated the runs. Rotavirus disease of Caco-2 cells diminishes sucrose-isomaltase movement and apical expression without enterocyte obliteration, proposing the association of trafficking instruments. So also, contamination of youthful rabbits or mice with rotavirus diminishes disaccharidase action [30]. What's more, NSP4 connected exogenously is known not Ca2+ discharge from intracellular stores and plasmalemmal Ca2+ inundation through a phospholipase C-subordinate instrument. This NSP4-intervened Ca2+ activation can bolster the runs by impacting Ca2+-subordinate epithelial procedures, for example, particle transport, obstruction capacity or cytoskeletal control. In reality, rotavirus has been exhibited to increment paracellular porousness in Caco-2 cells.

What's more, NSP4-intervened Ca2+ preparation may trigger the arrival of amines/peptides and the arrival of cytokines, prostaglandins and responsive oxygen species, which can alone or aggregately initiate the enteric nervous system (ENS) [31]. This was further affirmed with studies exhibiting that net rotavirus-intervened liquid transport was repressed by treatment of mice with medications that influence ENS capacity. Further, clinical studies in hospitalized, rotavirus-contaminated youngsters demonstrate that an enkephalinase inhibitor lessens loose bowels length [32]. Intracellular NSP4, in any case, is additionally known not intracellular

calcium levels through a PLC-autonomous component. Using NSP4-EGFP expression in HEK 293 cells, late studies show that intracellular NSP4 causes actin redesign in a calcium-subordinate way through diminished phosphorylation of the actin rebuilding protein cofilin. Regulation of subcortical actin elements and dysregulation of cofilin impacts film trafficking occasions and particle transport forms [33].

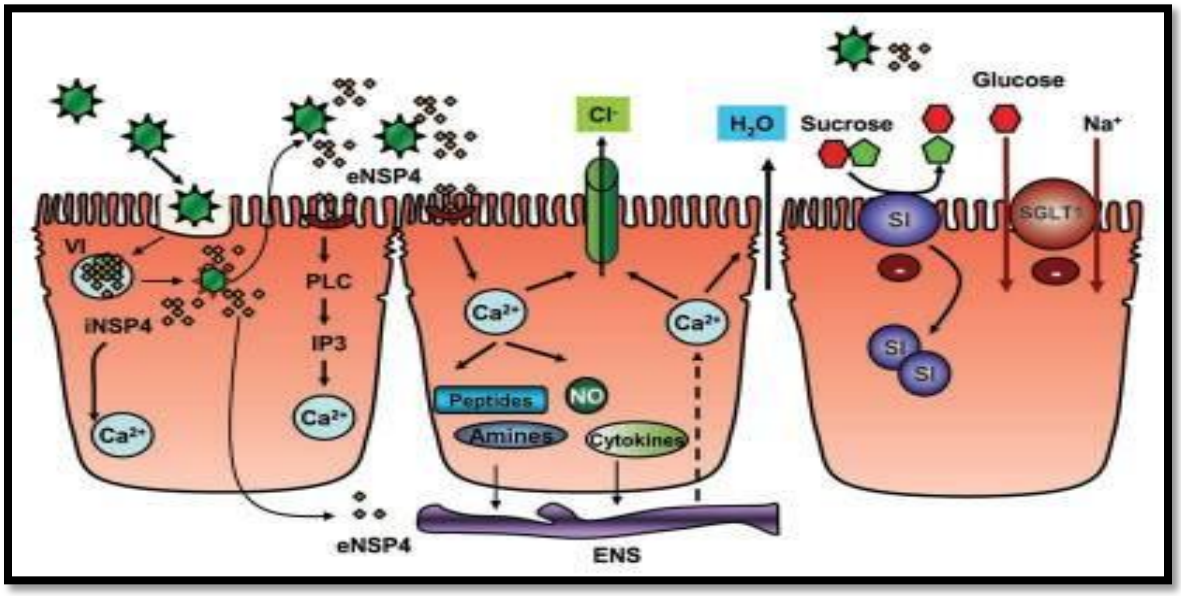


Figure 3: Components of rotavirus-interceded the runs. Rotavirus disease of enterocytes prompts infection passage, development of viroplasms (VI) and arrival of the infection and its poison, NSP4. Intracellular NSP4 (iNSP4) actuates an expansion in intracellular Ca2+ principally through discharge from er and a PLC-free system. NSP4 discharged from the apical side increments intracellular calcium levels through a receptor-intervened PLC-subordinate component. The expansion in calcium by NSP4 disturbs microvillus cytoskeleton and additionally hindrance capacity, prompting an increment in the paracellular stream of water and electrolytes, bringing on looseness of the bowels. The NSP4 affected expansion in Ca2+ levels can likewise expand Cl− emission straightforwardly through a CFTR-free component and can bring about arrival of amines, peptides, cytokines and receptive oxygen species, which can invigorate the enteric sensory system by implication to build Cl− discharge. The basolateral arrival of NSP4 may likewise fortify ENS. Maldigestion of starches because of a decline in surface levels of sucrase-isomaltase and diminished capacity of SGLT-1 gives off an impression of being a noteworthy system basic loose bowels brought on by rotavirus disease. eNSP4 is extracellular NSP4.

The Cl− secretory part basic the pathogenesis of rotavirus-related the runs is mind boggling, involved both ace and against secretory segments. Not at all like the absolutely secretory looseness of the bowels brought on by CT, rotavirus diseases just respectably expands luminal Cl−concentration. An expansion in luminal Cl−concentrations could be a result of diminished ingestion and/or expanded discharge. Early studies showed that NSP4 can bring about looseness of the bowels in youthful mice, which is connected with Ca2+ assembly and potentiation of cAMP-ward liquid emission. Strangely, in CFTR-inadequate mouse pups, NSP4 keeps on bringing about the runs precluding the contribution of CFTR in liquid aggregation. Suddenly, rotavirus disease of rabbits really animates Cl− assimilation in intestinal brush outskirt layer segregated from villus cells and does not change Cl− secretory reactions in tomb cells. In any case, the net Cl− secretory reaction is feeble, recommending that NSP4 applies both secretory and hostile to secretory activities to breaking point general Cl− discharge. More inside and out studies are required to portray the cell components hidden rotavirus related Cl− secretory reactions. The potential part of apical Cl−/HCO3− exchangers, CLC chloride channels and key flagging occasions in the pathogenesis of rotavirus disease would be of most extreme interest.

2.4 Replication

The rotavirus virion first joins to the objective cell; numerous strains tie cell surface sialic acids through VP8* (created by cleavage of VP4 into VP5* and VP8*) at the tips of the virion spikes. Non-clathrin-, non-caveolin-interceded endocytosis conveys the virion to the early endosome. There, decreased calcium fixations are thought to trigger uncoating (loss of VP7) of the triple-layered molecule (TLP) and film infiltration by VP5*. Loss of the external capsid and arrival of the double layered molecule (DLP) into the cytosol initiates the inner polymerase complex (VP1 and VP3) to translate topped positive-sense RNA ((+)RNAs) from each of the 11 twofold stranded RNA (dsRNA) genome portions. (+)RNAs serve either as mRNAs for amalgamation of viral proteins by cell ribosomes or as formats for combination of negative-sense RNA ((–)RNA) amid genome replication [34]. Non-structural protein 2 (NSP2) and NSP5 communicate to shape huge incorporations (viroplasms) that sequester segments required for genome replication and the gathering of subviral particles. Genome bundling is started when VP1 (and, apparently, VP3)

tie the 3′ end of viral (+)RNAs. It is as of now believed that communications among the 11 (+)RNAs drive arrangement of the 'grouping complex'. Buildup of the inward capsid protein, VP2, around the grouping complex triggers dsRNA blend by VP1. The middle capsid protein, VP6, then collects onto the beginning center to shape the DLP. Get together of the external capsid is not surely knew; the present model suggests that communication with the rotavirus transmembrane protein, NSP4, initiates DLPs and the external capsid protein VP4 to the cytosolic face of the endoplasmic reticulum (ER) film. Through an unclear instrument, the DLP–VP4–NSP4 complex buds into the ER. Resulting evacuation of the ER layer and NSP4 allows get together of the ER-occupant external capsid protein, VP7, and development of the TLP (Figure 4). Discharge from the contaminated cell opens the virion to trypsin-like proteases of the gastrointestinal tract, bringing about the particular cleavage of VP4 into VP5* and VP8* to create the completely irresistible virion.

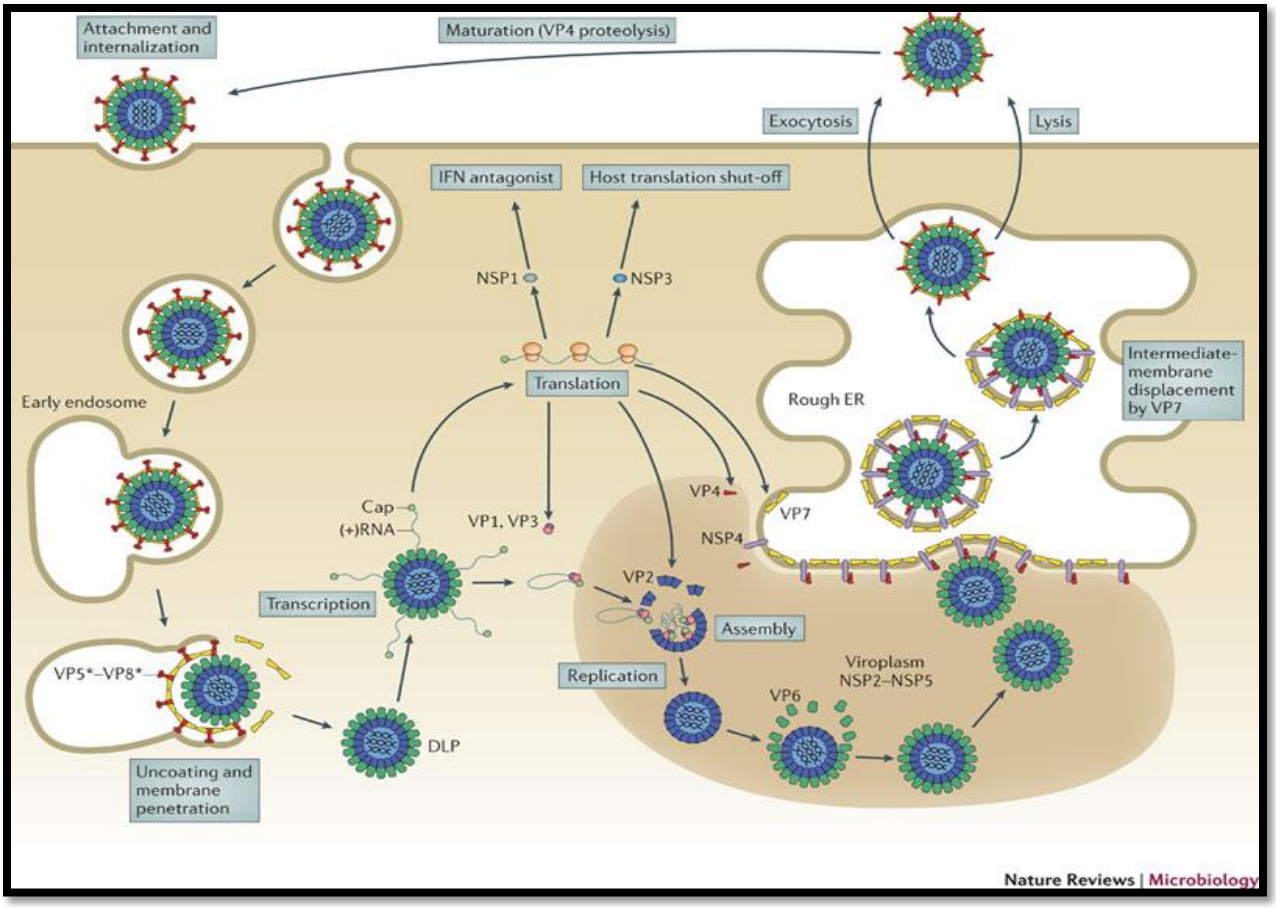


Figure 4: Rotavirus replication cycle [34].

2.5 Classification

Rotaviruses constitute the variety Rotavirus, one of the 15 genera of Reoviridae family which is subdivided into the sub-groups of the Sedoreovirinae (genera Cardoreovirus, Mimoreovirus, Orbivirus, Phytoreovirus, Rotavirus, Seadornavirus) and the Spinareovirinae (genera Aquareovirus, Coltivirus, Cypovirus, Dinovernavirus, Fijivirus, Idnoreovirus, Mycoreovirus, Orthoreovirus, Oryzavirus). As per the serological reactivity and hereditary variability of VP6, no less than 7 diverse groups, additionally termed species, are separated (termed RVA-RVG) [35]. Out of the seven groups, groups A, B and C rotaviruses are known to infect human, Group A being the real reason for rotavirus related grimness and mortality. Bunch D, E, F and G rotavirus have never been found to taint people and are limited to non-people, particularly aves.

Further characterization of rotaviruses is finished with a G/P-genotyping framework that depends on the examination of (i) Glycoprotein VP7 (G sort) and (ii) Protease-sensitive protein VP4 (P sort) qualities by reverse transcriptase polymerase chain reaction (i.e., RT-PCR writing) or by cDNA sequencing [6].

The RVA species includes no less than 27 G sorts (as indicated by the nt succession of VP7) and 37 P sorts (as per the nt arrangement of VP4) [7]. For G sorts, serotypes and genotypes are synonymous, e.g. G1, G2, and so forth. For P sorts, there are numerous more P genotypes than reference sera deciding P serotypes: hence, a twofold classification has been presented, e.g. P1A[8] assigning the P serotype 1A and P genotype 8, and so forth. An extensive, nt arrangement based order containing the complete genome has been presented for RVAs, in which the VP7–VP4–VP6–VP1–VP2–VP3–NSP1–NSP2–NSP3–NSP4–NSP5/6 genotypes are recognized and separated by cut-off purposes of nt grouping personalities [36]. Most usually detached G and P sorts are G1, G2, G3, G4, G9 and P[4], P[8] separately. Concentrates on uncover the presence of more than 70 diverse G-P mixes. Out of these, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] are most normally recognized G-P blends and records for around 74% of rotavirus contaminations all around [37].

Alongside the basic strains, countless studies have archived the presence of numerous uncommon and unprecedented strains in people. The use of cutting edge atomic strategies, for example, RT-PCR and sequencing investigation have brought about exponential increment in the part of exceptional and recently distinguished strains. The advancement of rotavirus results from four systems: point transformation, interspecies transmission of fractional or entire infection, re-assortment occasions amid co-disease of two distinctive infections in a typical host and quality reworking that ideally targets non-structural protein (NSP) coding fragment of the genome. These components work independently or in mix with each other bringing about the differing gathering of rotaviruses. Table 2 exhibits the tremendous level of genomic assorted qualities among RVAs co-coursing in various populace gatherings and creatures at different times. As of late, comparative extensive genotype separations for RVBs and RVCs have begun to be set up.

Table 2: Genotypes of species A rotaviruses [38].

RV protein	Percent identity[a]	Number of genotypes[b]	Genotype (acronym underlined)
VP7	80	27G	Glycosylated
VP4	80	37P	Protease-sensitive
VP6	85	18I	Inner capsid
VP1	83	9R	RdRp[c]
VP2	84	9C	Core protein
VP3	81	8M	Methyltransferase
NSP1	79	19A	Interferon Antagonist
NSP2	85	10N	NTPase
NSP3	85	12T	Translation enhancer
NSP4	85	15E	Enterotoxin
NSP5	91	11H	PHosphoprotein

a Nucleotide percent identity cut off value defining genotypes.

b October 2013 (Rotavirus Classification Working Group, 6th meeting, Valencia/Spain).

c RNA-dependent RNA polymerase.

2.6 Vaccines

Execution of a successful rotavirus immunization system should consider the geological variety of pervasive strains. The proceeded with recognizable proof of the most widely recognized G and P serotypes for incorporation in immunizations is an imperative need. After the presentation of an immunization hopeful, observing of circling strains might be essential, as antibody weight may prompt the determination of novel rotavirus strains.

Endeavors to build up an immunization against human rotavirus started in the mid 1980s. Introductory endeavors utilized a "Jennerian" approach (in reference to Edward Jenner's cowpox immunization against smallpox) to inoculate youngsters against rotaviruses, which regularly taint creatures. A few critical discoveries rose up out of the principal immunization ponders. The RIT4237 cow-like rotavirus immunization hopeful was sheltered and was found exceedingly compelling (more prominent than 80%) in counteracting extreme looseness of the bowels in Finnish kids, however altogether less successful in clinical trials in African and Latin American youngsters. The RIT4237 antibody was less powerful (as have been all ensuing immunization

applicants) at keeping any the runs than at counteracting serious disease. At long last, and most strangely from the outlook of the part of immunization serotype in insurance, the cow-like RIT antibody was successful in spite of the way that it was antigenically befuddled with all coursing human rotavirus strains. As a result of its disappointment in clinical trials in Africa, the RIT4237 competitor was not sought after.

These underlying studies were trailed by a more managed exertion from examiners at the National Institutes of Health (NIH) and Wyeth Pharmaceuticals to build up an enhanced creature rotavirus-based antibody. A simian rotavirus (RRV) was at first assessed as a monovalent applicant that gave off an impression of being compelling in preparatory trials, yet ensuing studies uncovered decreased viability. This disappointment was proposed to reflect contrasts between the serotype of the RRV immunization (G3) and coursing human strains at the season of the trial. Likewise, the monovalent RRV strain had a lot of remaining reactogenicity, essentially as fever. To go around the conceivable serologic issues intrinsic in a monovalent antibody, an altered procedure was utilized, in which the quality encoding VP7 from RRV (which was a G3 strain) was supplanted with qualities encoding human G1, 2 and 4 VP7s and a tetravalent immunization containing G1,2,3 (from the first RRV) and 4 was assessed (Kapikian, 2001). This antibody, called RotaShield™ or RRV-TV, was assessed in a broad arrangement of security and adequacy examines in the US, Finland and Venezuela, which all showed it was very successful (80%–100%) in forestalling serious diarrheal malady [39].

In spite of the fact that the tetravalent immunization was exceptionally compelling, the immunologic premise for this adequacy was misty. Of note, balance reactions to the 4 G serotypes contained in the antibody were much lower than the clear viability rates of the immunization. To clarify this obvious disagreement, it has been hypothesized that the essential point of preference of multivalent rotavirus antibodies is not their serotypic assorted qualities yet rather their expanded capacity, contrasted with monovalent builds, to help the resistant reaction on the second or third organization. Regardless, the RotaShield™ antibody was judged to be protected and viable in a few vital stage 3 clinical trials and was authorized for general use in youngsters 2 to 6 months of age in the US in August, 1998 with elevated requirements that the threats of rotavirus disease would soon be disposed of.

Roughly 600,000 newborn children in the US got RRV-TV before its usage stopped in July 1999, when it was accounted for that the primary measurement of RRV-TV was connected with a generous expanded relative danger (no less than 25-fold) of intussusception inside the initial 10 days after organization (Centers for Disease Control and Prevention, 1999). The component that underlies the relationship between RRV-TV and intussusception is obscure, however has been proposed to be particular to the RRV strain, since wild-sort rotaviruses and other live constricted antibodies have not been reproducibly connected with an expanded rate of intussusception. Construct principally in light of the expansion in relative danger, Rotashield was judged to be hazardous for routine utilize and pulled back from business produce. It took an additional 7 years before new rotavirus immunization applicants were accessible; amid this seven 7-year rest, rotavirus brought on horribleness and mortality proceeded with unabated. Numerous moral inquiries concerning the propriety of killing the accessibility of RRV-TV immunization remain, particularly for kids in less-created parts of the world, where the risk:benefit proportion for usage of RRV-TV was altogether different than in the US.

Luckily, scrutinize on rotavirus antibodies proceeded after the unforeseen issues with the RRV-TV and in 2006, 2 new rotavirus immunizations were authorized in the US, the European Union, and in addition numerous nations in Central and South America [40] (Table 3). One of these new immunizations speaks to an option approach. For this situation, an ox-like rotavirus strain (WC3), secluded in the US, was utilized as a spine to make a pentavalent immunization that contained 5 separate infections that communicated either human G1, 2, 3 or 4 VP7s and a human P(8) VP4 on the ox-like WC3 spine [41]. The WC3 strain was at first concentrated on as a stand-alone monovalent competitor (much like the RIT immunization). It was observed to be fittingly weakened however clinical trials yielded changing viability rates, which prompted the alteration and incorporation of the different human G and P sorts. The pentavalent WC3-based immunization is fabricated by Merck and is advertised under the exchange name RotaTeq™. On account of the wellbeing issues with RRV-TV, enrollment trials required right around 70,000 newborn children. In these trials, which were principally yet not only did in the US and other created nations, the antibody was exceptionally strong with security rates against any rotavirus the runs of 74%, against looseness of the bowels requiring a doctor visit of 87% and against serious rotavirus ailment of as high as 100%. RotaTeq's adequacy rates did not seem, by all

accounts, to be influenced by breastfeeding and organization of this immunization did not meddle with insusceptible reactions incited by different antibodies [42]. Above all, the antibody was sheltered and not connected with intussusception. Truth be told the rates of intussusception were fairly lower in antibody beneficiaries. Late postliminary, post-licensure considers from the CDC have not unveiled rate of intussusception that is more noteworthy than anticipated for antibody beneficiaries (Centers for Disease Control and Prevention, 2007).

Table 3: Comparison of the Two Licensed Rotavirus Vaccines [40].

	Rotateq™	**Rotarix™**
Manufacturer	Merck Vaccine Division	GlaxoSmithKline
Genetic Backbone	Bovine Rotavirus-WC3	Human rotavirus-89-12
Composition	5 human; bovine reassortant	Single human rotavirus
Genotypes	G1,2,3,4 and [P8]	G1 [P8]
Dosage schedule	3 doses @ 2, 4, & 6 months of age	2 doses @ 2 & 4 months of age
Administration	Oral	Oral
Presentation	Liquid	Lyophilized-reconstituted
*– Protection against severe disease	85% (72–92)	95% (91–97)*–
Virus shedding	9%	50% or more
Intussusception	No	No

The sub-atomic premise for the weakening of the WC3-based antibody is not in the blink of an eye known. Truth be told, the premise for host range limitation of rotaviruses all in all is ineffectively caught on. It is accepted, yet not demonstrated, that an immunization that is constricted on the premise of host extent limitation will be hereditarily steady. RotaTeq is given in a 3-measurement timetable and preparatory information demonstrate that no less than 2 dosages are required to produce huge levels of insurance. Of course for an immunization taking into account a creature rotavirus seclude, antibody shedding has been accounted for as occasional and at a low level. The immunization had all the earmarks of being successful in averting extreme sickness brought about by an assortment of rotavirus serotypes, including G9 strains,

despite the fact that a G sort part is not present in the real antibody. Extra proof supporting the thought that serotype particular insusceptibility is not exclusively in charge of insurance is the finding (as was additionally seen with the Rotashield antibody) that sort particular balance reaction rates taking after inoculation were much lower than the assurance rates saw in clinical trials. RotaTeq was authorized in the US in 2006 and by late 2008 its impact on reported diarrheal malady in youngsters were surveyed in an across the nation study (Prevention, 2008). The CDC evaluated that immunization was connected with a considerable postponement in the yearly onset of the rotavirus season and a more noteworthy than half decline in rotavirus action. This generous decrease was more huge in light of the fact that it occurred amid a period when just a minority of the vulnerable kids had been given the immunization, so it may have the capacity to lessen transmission and give 'crowd safety' (group based) and also singular resistance.

A live constricted human rotavirus immunization was authorized in 2006 under the exchange name Rotarix™. This destructive G1P [43] human rotavirus strain was passaged for different rounds in monkey kidney cell societies to accomplish constriction. The underlying passaged material had leftover destructiveness, however taking after ensuing extra sections and plaque sanitization, completed by GlaxoSmithKline, a profoundly constricted item was accomplished. Similarly as with the Merck immunization (RotaTeq), the sub-atomic premise for the lessening of the Rotarix antibody is obscure, in spite of the fact that an arrangement examination with its wild-sort guardian strain could recognize the qualities changes that are connected with constriction. In spite of the fact that there has been no immediate examination amongst RotaTeq and the GlaxoSmithKline antibody, this human rotavirus immunization is evidently shed in significantly more noteworthy sums than RotaTeq, the ox-like determined immunization. This would likely demonstrate a higher probability of transmission from immunized to unvaccinated contacts. In any case, better comprehension of the hereditary premise of its weakening and the level of its hereditary security taking after transmission would associate improvement of future antibodies.

The basis fundamental the improvement of Rotarix was that a solitary regular rotavirus contamination, either symptomatic or asymptomatic, gives defensive insusceptibility against resulting serious sickness, regardless of serotype. In this way, it appeared to be intelligent to

anticipate that a weakened human rotavirus strain may do likewise. In light of earlier security worries with the RRV-TV, extensive scale (>60,000 kids) wellbeing and adequacy trials were required for licensure. Not at all like RotaTeq, these were completed principally, however not solely in nations in Central and South America. Rotarix requires just 2 dosages, likely on the grounds that it is better adjusted to replication in the human GI tract than the ox-like based antibody and it can be directed at a measurement around 100-fold lower than that of RotaTeq. The vast scale security study directed in Latin America demonstrated no relationship amongst Rotarix and intussusceptions [44]. Viability trials in Latin America and Europe demonstrated the immunization to be profoundly compelling. In a subset of the huge enlistment security study partner, the antibody was 85% successful against averting extreme loose bowels and 100% compelling against the most serious cases. Curiously, in spite of the monovalent way of the immunization, it was compelling (92%) against homotypic G1 strains and 88% powerful against heterotypic G3, 4 and 9 strains. In this study, viability against G2 strains (41%) was not critical but rather ensuing meta-investigation concentrates on and other adequacy examines from Europe indicated considerable (81%) adequacy against G2P(4) strains. Late 2-year viability information for Rotarix have demonstrated that Rotarix does not meddle with other routine adolescence inoculations (Rodriguez et al., 2007). Since various infection side effect scoring frameworks were utilized by Merck and GlaxoSmithKlein amid their clinical trial programs, it is for all intents and purposes difficult to straightforwardly look at the efficacies of RotaTeq and Rotarix, albeit every immunization is profoundly compelling. In any case, there are waiting suspicions that Rotarix is less viable against G2 strains and that this relative inadequacy may, under a few circumstances, produce issues.

A few third era rotavirus immunizations are being developed in light of conceivable security issues connected with the of RotaTeq and Rotarix; as a result of this few gatherings are seeking after inactivated infection or recombinant infection like-molecule methodologies. Parenteral inoculation with inert infection has demonstrated successful in creature models yet no evidence of guideline for this methodology exists for people. Likewise, parenteral or intranasal vaccination with recombinant nonreplicating infection like molecule antibodies have been viable in all creature models tried, and these applicant immunizations are prepared for stage 1 testing in people. Another justification for the advancement of extra immunization applicants is cost—

rotavirus antibody will never be completely moderate in the poorest nations until immunization makers in the creating scene can contend with expansive pharmaceutical organizations.

There are various critical essential and useful issues to be determined concerning rotavirus antibody advancement yet maybe the absolute most imperative one is to figure out if RotaTeq and/or Rotarix are viable in exceptionally poor districts in Asia or Africa. Different immunizations, particularly orally regulated antibodies, have been found to have enormously reduced adequacy in certain exceptionally poor districts of India and Africa. As of now, under the sponsorship of the Seattle-based philanthropic association PATH (in the past called Program for Appropriate Technology in Health) and the backing of the Gates establishment, the viability of Rotarix and RotaTeq is being considered in parts of Africa as well as Asia. The consequences of these clinical trials are significantly expected. Another vital issue is to figure out if the confined planning of organization of the principal dosage of these antibodies will restrain their value in any nation. A few kids in the US are not profiting from rotavirus immunization in light of the fact that the main dosage should be regulated by a most extreme of 2 months of age; it not clear if these planning confinements are reasonable for creating nations. Immunization security in youngsters with immunodeficiencies likewise needs to observed; instances of unending disease happened in children with severe combined immunodeficiency (SCID) that got the antibody before they were determined to have this issue. This result is not startling as it has happened with other live lessened antibodies in SCID babies, however we have to set up approaches to oversee and keep these circumstances.

A sensible objective for a rotavirus immunization is to copy the level of assurance against malady that takes after common disease. In this way, antibody program goals incorporate the aversion of moderate to serious infection however not as a matter of course of mellow malady connected with rotavirus. A viable rotavirus immunization will unmistakably diminish the quantity of kids admitted to the doctor's facility with drying out or found in crisis offices yet ought to likewise diminish the weight on the honing essential consideration expert by decreasing the quantity of office visits or phone calls because of rotavirus gastroenteritis. At long last, compelling rotavirus immunizations are most required in asset poor nations, where mortality connected with rotavirus is high.

Table 4: Rotavirus vaccines that are regionally used, recently licensed, or in development [43].

Name	Composition	Route of administration	Organization/Company	Stage of development
Lanzhou Lamb Rotavirus (LLR)	Live attenuated lamb rotavirus strain, G10P[12]	Oral	Lanzhou Institute of Biological Products, China	Licensed for use in China
Rotavin-M1	Live attenuated human rotavirus strain, G1P[8]	Oral	POLYVAC, Vietnam	Licensed for use in Vietnam
ROTAVAC	Live attenuated neonatal rotavirus strain, G9P[11] (aka 116E)	Oral	Bharat Biotech, India	Recently licensed for use in India; pursuing WHO pre-qualification
LLR reassortants	Live attenuated lamb-human reassortant rotavirus strains, G2, G3, G4	Oral	Lanzhou Institute of Biological Products, China	Phase III
RotaShield	Live attenuated rhesus-human reassortant rotavirus strains, tetravalent	Oral	International Medica Foundation, USA	Phase II complete, Phase III pending

2.7 Detection

Determination of rotavirus contamination depends on the distinguishing proof of rotavirus in excrement or suspension of rectal swab gathered right on time in the ailment. The infection in stool might be straightforwardly shown by electron microscopy, polyacrylamide gel electrophoresis with silver stain. Human rotavirus can likewise be confined from feces tests in essential monkey kidney cells. Fast serological test include the utilization of latex agglutination packs while corroborative test with Enzyme Linked Immunosorbent Assay (ELISA) test for rotavirus particular antigens is additionally utilized [45] [46].

Rotavirus is discharged in substantial numbers in the excrement (>106 particles/g defecation) and in this way can be effectively recognized on electron microscopy of feces tests which is a standout amongst the most particular tests for analysis [47]. Direct EM examination of stools for rotavirus has an affectability of 80-90 percent. However EM requires costly gear and prepared staff and in this way can't be utilized as a part of field studies. Different strategies like immunoelectroosmophoresis and altered supplement obsession test were created, yet they needed affectability. Tailing this, numerous quick and practical measures like latex agglutination (LA), enzyme immuno assay (EIA) and polyacrylamide gel electrophoresis (PAGE) were utilized for determination [48] [49]. Particular ELISA tests in view of monoclonal antibodies have been produced [50]. New strategies like blot hybridization utilizing radio named cDNA tests and reverse transcriptase - polymerase chain reaction (RT-PCR) are presently being utilized as corroborative techniques for identifying rotavirus in feces tests [51]. RT-PCR has been observed to be an exceptionally touchy and particular technique for conclusion of rotavirus in feces test from patients with intense bowel movements.

The most generally utilized research facility analytic methods are ELISA and RT-PCR.

ELISA: Transmission electron microscopy (EM) was the strategy at first used to distinguish infection in fecal and intestinal biopsy tests, and remains the standard to which rotavirus analytic tests are analyzed. Rotaviruses are for the most part exceptionally hard to recognize in vitro; consequently, cell society is not routinely utilized for discovery and analysis. The compound immunoassay (EIA) is a basic, exceedingly touchy strategy for the identification of rotavirus antigen, and is appropriate for investigation of expansive quantities of tests.

It uses monoclonal antibodies in a strong stage sandwich sort EIA. Plastic microtiter wells are covered with a monoclonal neutralizer coordinated against the result of the 6th viral quality (VP6), which is the gathering particular antigen for all known human rotaviruses. An aliquot of fecal suspension is added to the well and brooded all the while with a hostile to rotavirus monoclonal immunizer conjugated to horseradish peroxidase, bringing about the rotavirus antigen being sandwiched between the strong stage and compound connected antibodies. Urea peroxide and TMB are utilized as substrates for the chemical. The catalyst bound in the wells changes over the boring substrate to blue shading. The force of the blue shading is straightforwardly corresponding to the convergence of rotavirus antigen in the example.

RT-PCR: A touchy strategy, similar to reverse transcriptase polymerase chain reaction (RT-PCR), may uncover more rotaviruses. RNA extricated from stool examples is changed over to cDNA utilizing arbitrary hexamers as groundwork and further investigated on the premise of nearness of VP6 antigen (utilized for grouping of rotaviruses) utilizing particular primers.

CHAPTER 3

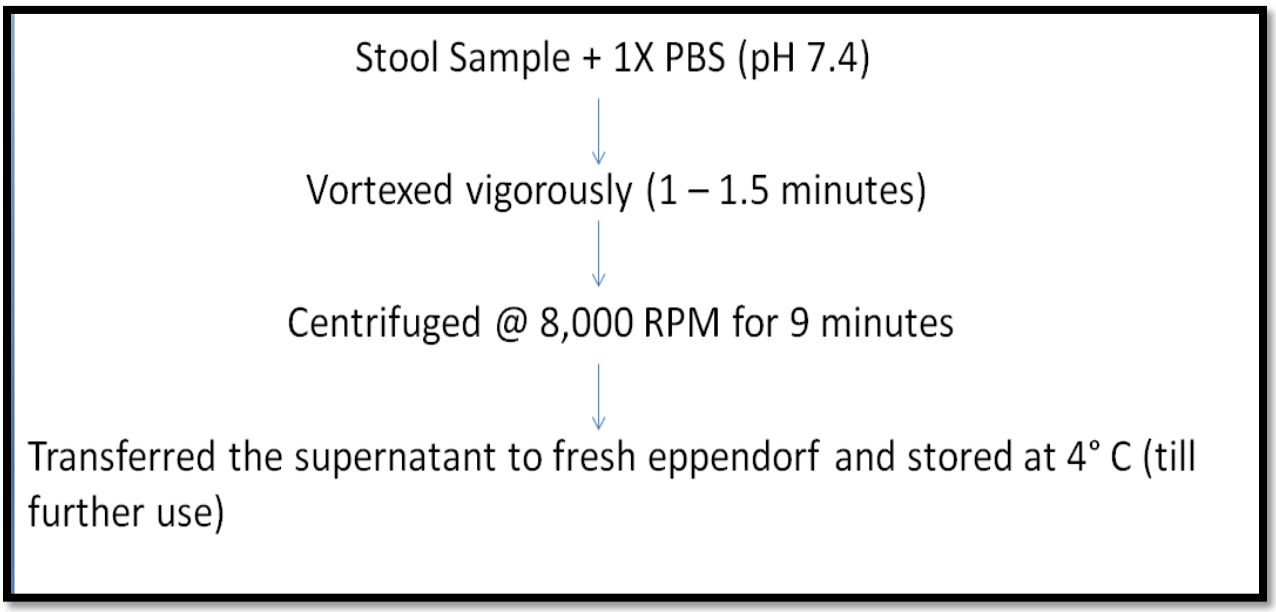
Materials and Methods

3.1 Stool Suspension/ Fecal Suspension Preparation

For isolation of viral particles from the amalgam of cell debris, bacterial cells, mucus cells etc. it is required to prepare the fecal suspension.

Materials Required: Collected stool samples (from IGMC, Shimla and Regional Hospital, Solan), 1X PBS (pH 7.4), vortex, centrifuge, eppendorfs, pipette, tips, spoons, cotton, gloves, autoclavable bag etc.

Protocol:



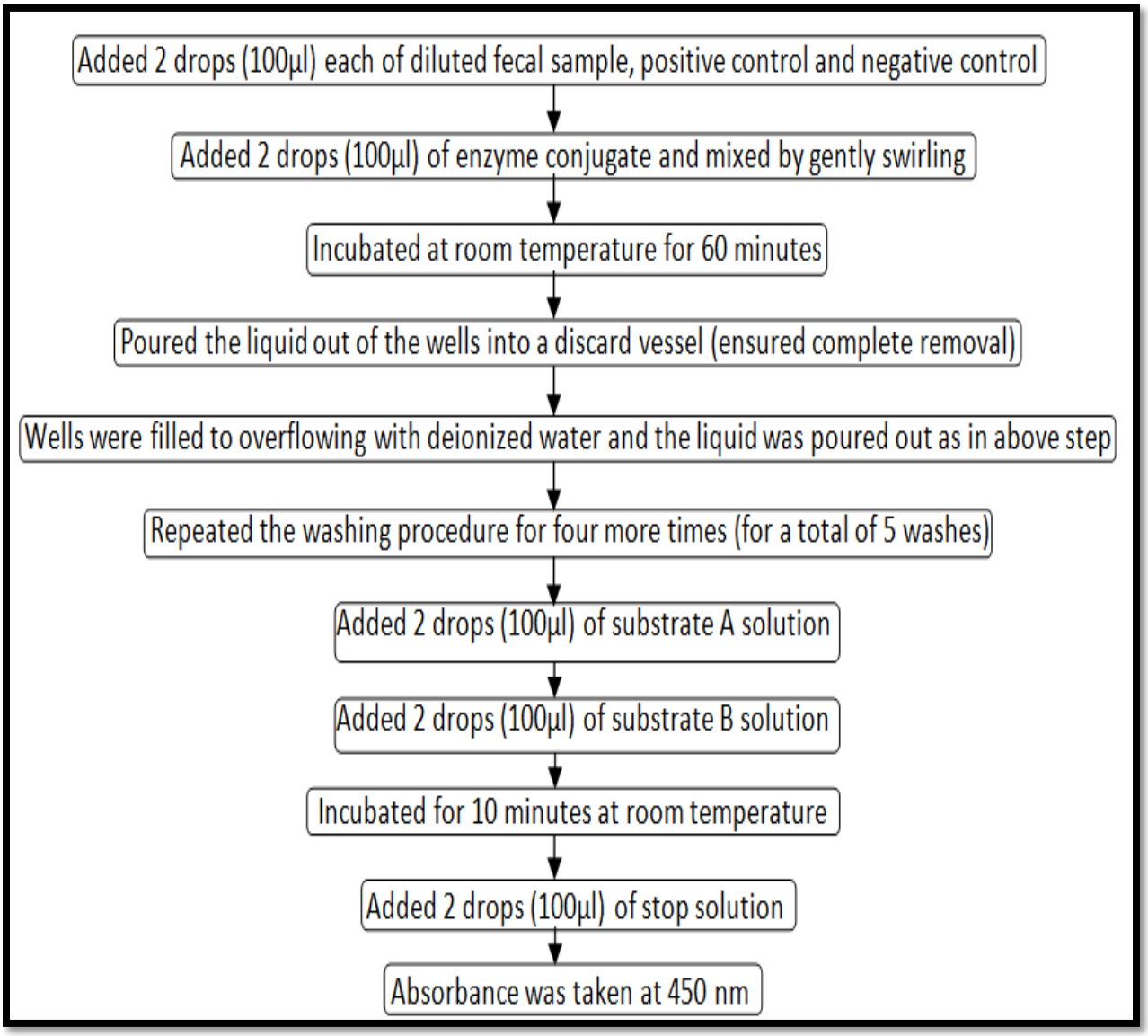
3.2 ELISA

The 10% fecal suspensions prepared (SS) were utilized for identification of rotavirus using the principle of 'sandwich ELISA' by means of the 'Premier Rotaclone' kits from Meridian Bioscience. The protocol followed was same as provided in the kit. The identification of the

rotavirus antigen VP6 is the basis of this preliminary test. The reason for doing ELISA is the specificity it offers.

Materials Required: Substrate A solution (urea peroxide), substrate B solution (TMB), enzyme conjugate, positive control (provided in the kit), microtiter plate, sample diluent, stop solution (contains 1N H_2SO_4), stool suspension samples, deionizd water, discard vessel, absorbent paper, microwell plate reader, pipette, tips, gloves etc.

Protocol:

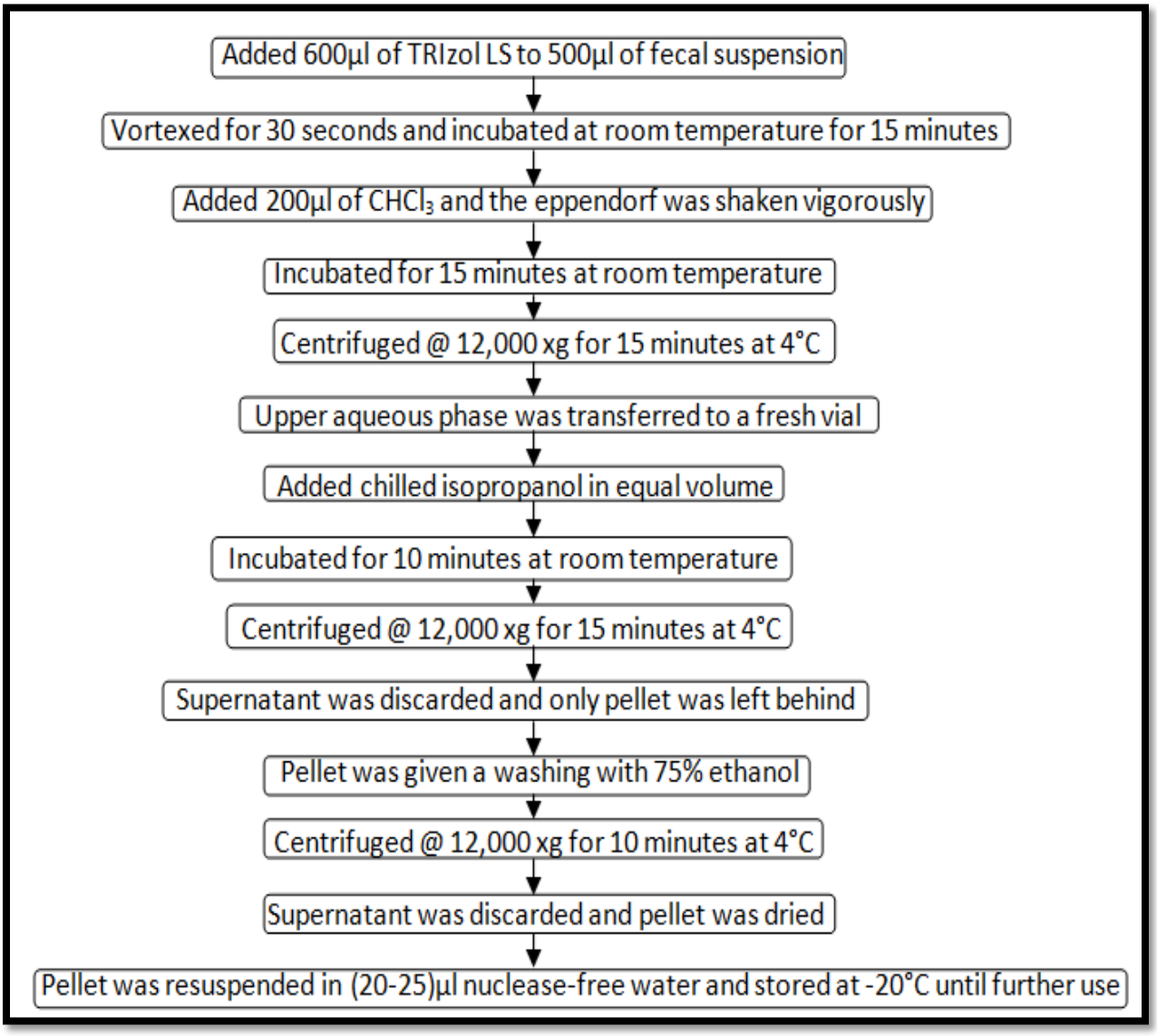


3.3 RNA extraction

Samples identified as 'positive' by ELISA are further needed for G/P genotyping by PCR – based methods. So in the process, RNA isolation was done of the rotavirus positive samples from the 10% fecal suspensions using TRIzol method.

Materials Required: Trizol LS reagent, $CHCl_3$, isopropanol, 75% ethanol (in DEPC – treated water), nuclease – free water, DEPC - treated tips and vials, vortex, centrifuge, stool suspension samples, pipette, tips, gloves etc.

Protocol:

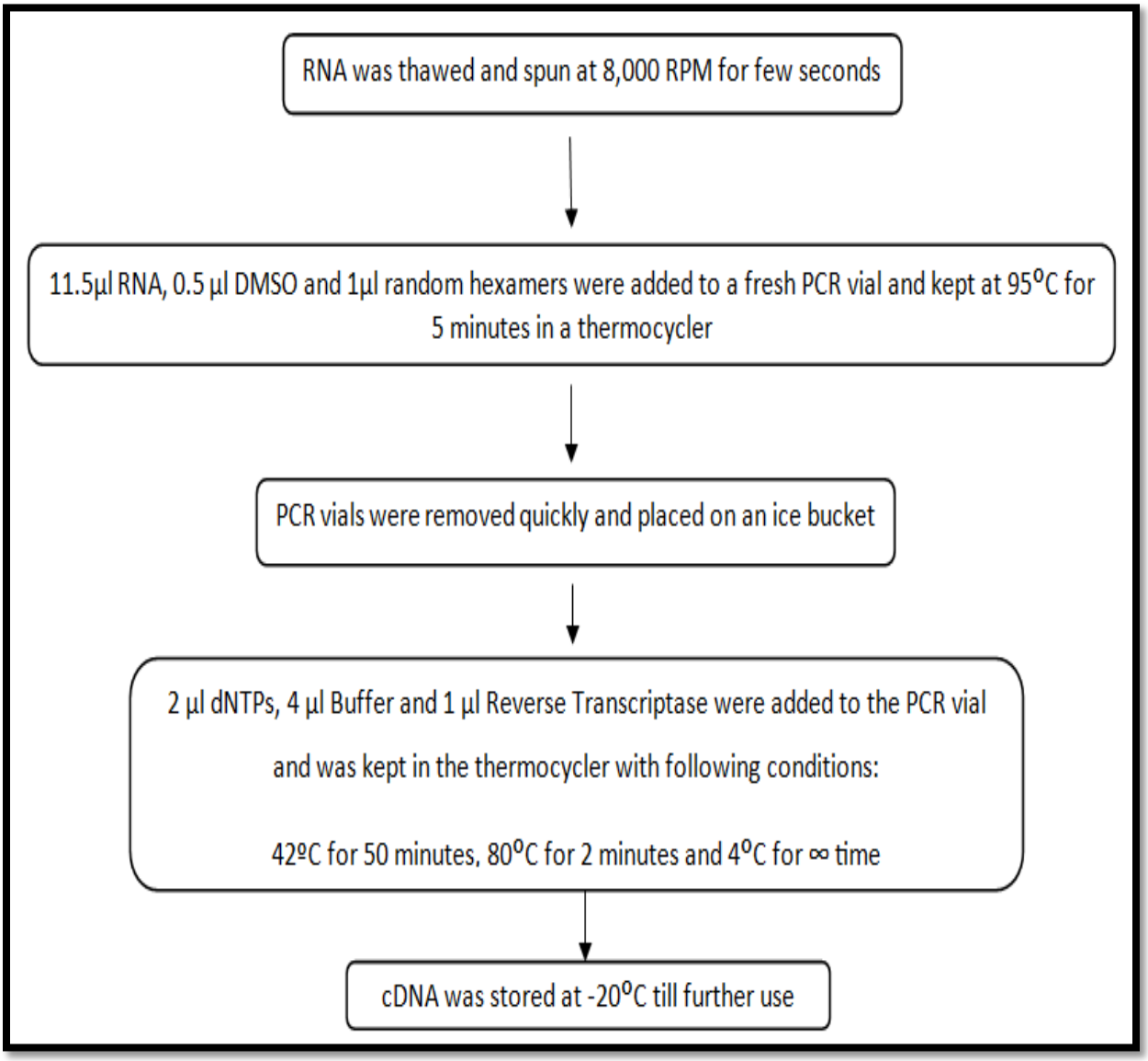


3.4 cDNA synthesis

RNA extracted in previous step needs to be converted to cDNA for further assistance in genotyping. As cDNA is more stable, it can be stored for a longer time in intact form compared to RNA.

Materials Required: RNA, dNTPs, DMSO, reverse transcriptase, buffer, random hexamers, ice bucket, PCR vials, pipette, tips, thermocycler etc.

Protocol:



3.5 PCR for VP6

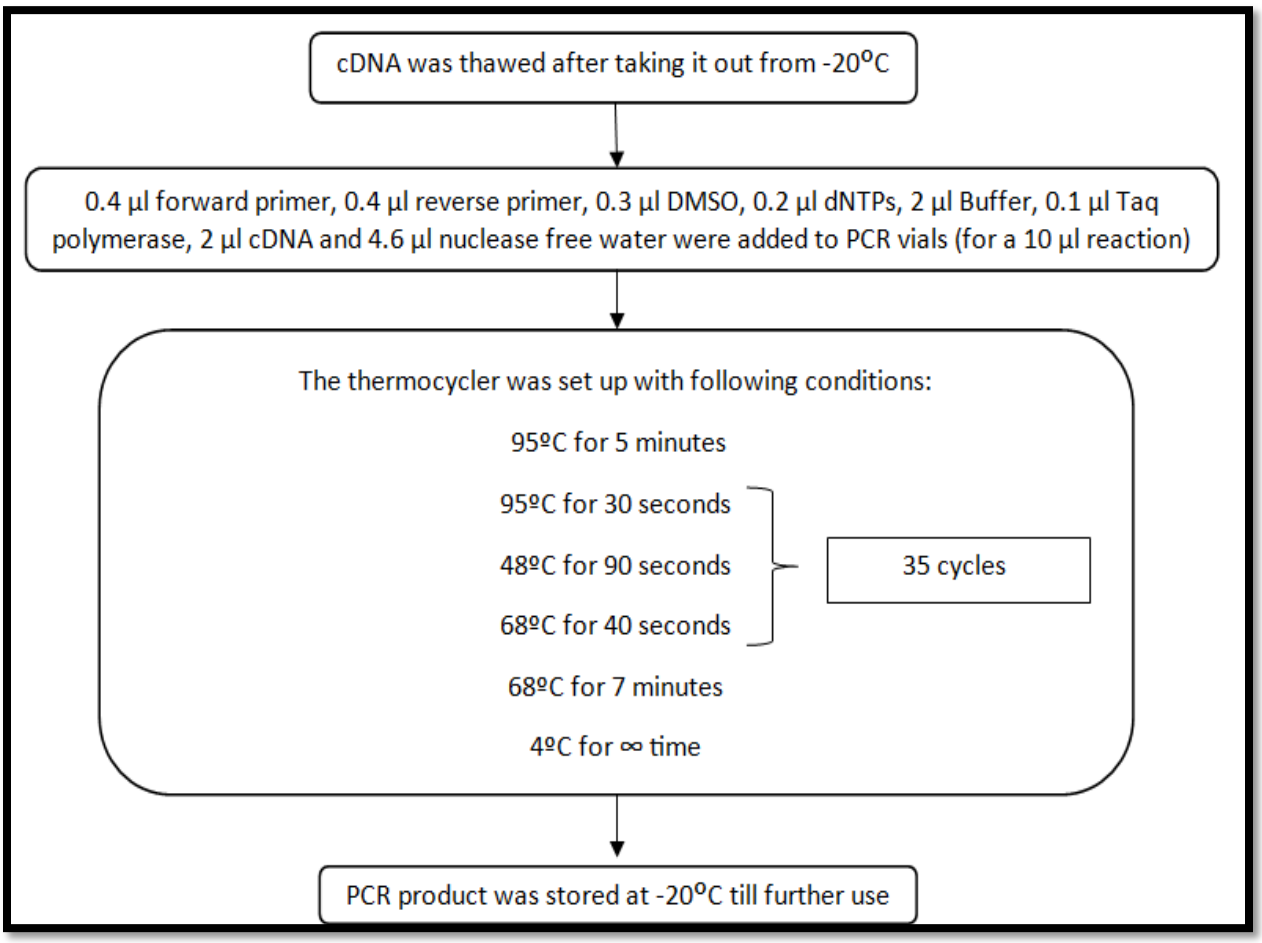
cDNA synthesised in previous step is used for identification of VP6 gene (227 bp) of group A rotaviruses by PCR. Another purpose is to check the integrity of previous steps (especially RNA isolation) or how well the previous steps were performed.

Materials Required: cDNA, dNTPs, DMSO, Taq polymerase, buffer, primers (forward and reverse), nuclease free water, ice bucket, PCR vials, pipette, tips, thermocycler etc.

Virus and primer	Sequence	Sense	Amplicon size (bp)	References
Group A rotavirus				
VP6 (F)	TTTGATCACTAAYTATTCACC	+	227	Mondal et al.
VP6 (R)	GGTCACATCCTCTCACTA	–		

Primer sequence refereed from Mondal et al. 2013 [52].

Protocol:

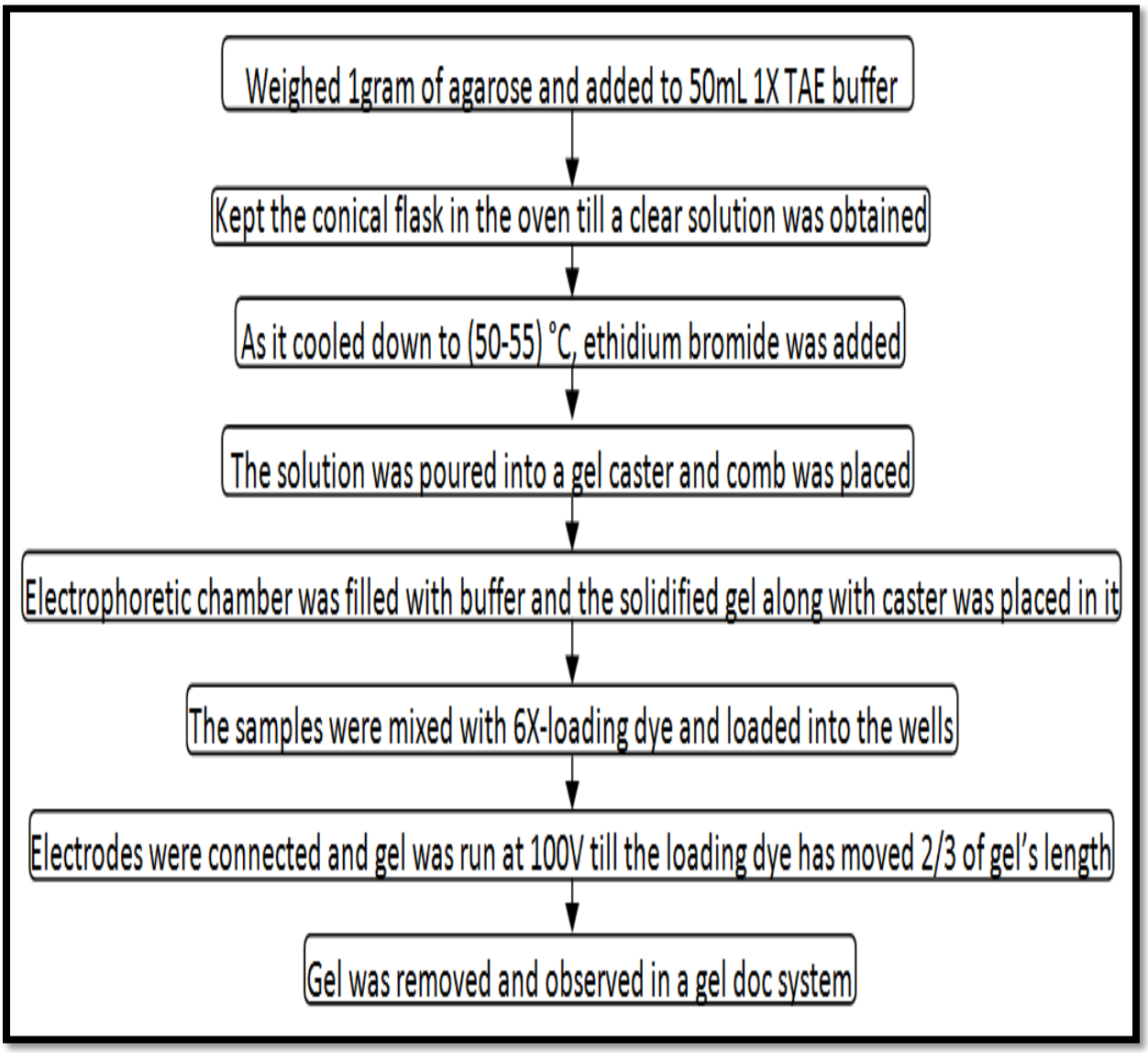


3.6 Agarose gel electrophoresis

The products of VP6 PCR were run on 2 % agarose gel along with 100 bp ladder.

Materials Required: RNA samples, agarose, 1X TAE, weighing balance, conical flask, measuring cylinder, oven, ethidium bromide, electrophoresis unit, combs, pipette, tips, loading dye, parafilm, gel doc etc.

Protocol:



CHAPTER 4

Results & Discussions

4.1 ELISA

4.1.1 Positive Results by Visual Determination

Any sample with blue color more intense than that of the negative is considered positive. Any sample with color equal to or less intense than the negative control is considered negative.

4.1.2 Positive Results by Spectrophotometric Determination

Specimens with absorbance units (A_{450}) greater than 0.150 are considered positive. Specimens with absorbance equal to or less than 0.150 are considered negative.

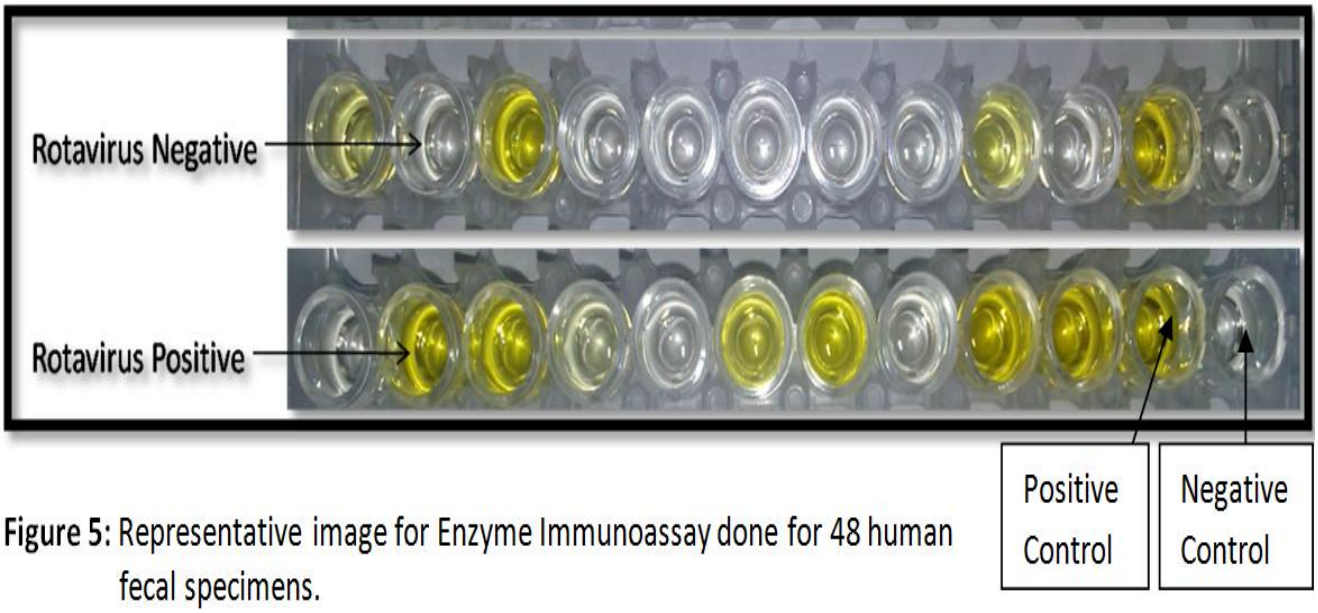


Figure 5: Representative image for Enzyme Immunoassay done for 48 human fecal specimens.

4.2 Agarose gel electrophoresis

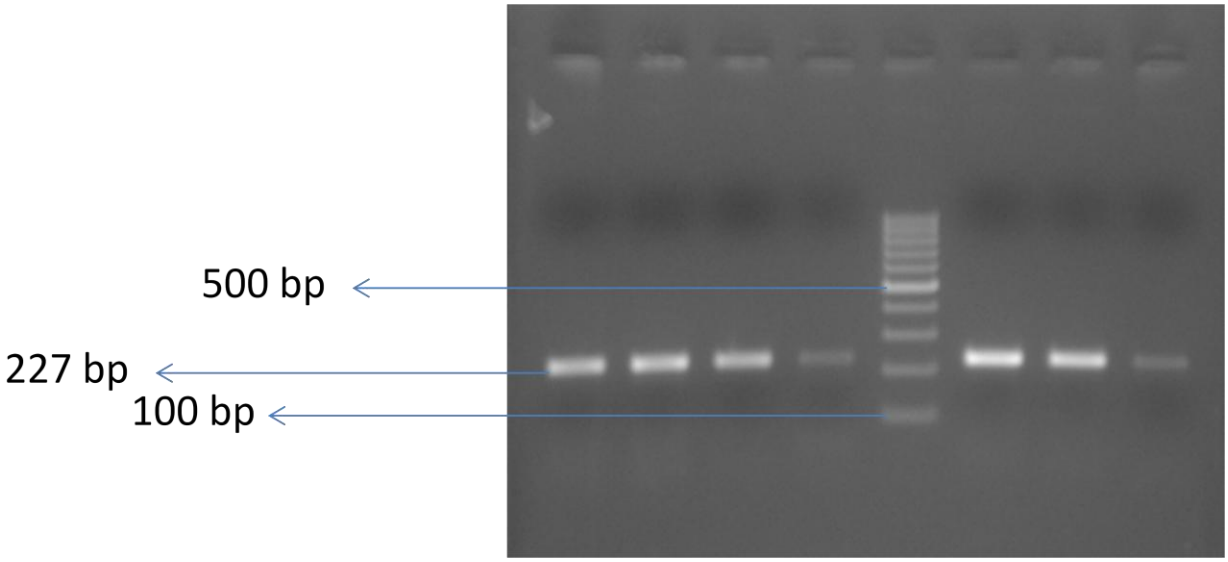


Figure 6: Representative image of 2% gel for VP6 PCR products. Presence of bands (227 bp) shows rotavirus positive samples.

4.3 Rotavirus positive samples

Table 5: Compiled table for results **a:** <5 years considered as Child and >=5 years as Adult. **b:** Along with absorbance for positive samples. **c:** On the basis of presence (yes)/absence (no) of bands on 2% agarose gel.

S. No.	Age Group [a]	ELISA [b]	VP6 PCR [c]
1	CHILD	-	No
2	CHILD	+ (0.318)	Yes
3	CHILD	-	No
4	CHILD	-	No
5	CHILD	-	No
6	CHILD	-	No

7	**CHILD**	**+ (0.470)**	**Yes**
8	**CHILD**	**+ (0.298)**	**Yes**
9	**CHILD**	**-**	**No**
10	**CHILD**	**-**	**No**
11	**CHILD**	**-**	**No**
12	**CHILD**	**-**	**No**
13	**CHILD**	**+ (0.653)**	**Yes**
14	**CHILD**	**-**	**No**
15	**CHILD**	**-**	**No**
16	**CHILD**	**-**	**No**
17	**CHILD**	**-**	**No**
18	**CHILD**	**-**	**No**
19	**CHILD**	**+ (0.210)**	**Yes**
20	**CHILD**	**+ (0.430)**	**Yes**
21	**CHILD**	**-**	**No**
22	**CHILD**	**-**	**No**
23	**CHILD**	**-**	**No**
24	**CHILD**	**-**	**No**
25	**CHILD**	**+ (0.204)**	**Yes**
26	**CHILD**	**-**	**No**
27	**CHILD**	**+ (0.376)**	**Yes**
28	**CHILD**	**+ (0.177)**	**Yes**
29	**CHILD**	**-**	**No**
30	**CHILD**	**+ (0.157)**	**Yes**
31	**CHILD**	**-**	**No**
32	**ADULT**	**-**	**No**
33	**ADULT**	**-**	**No**
34	**ADULT**	**-**	**No**
35	**ADULT**	**-**	**No**
36	**ADULT**	**-**	**No**

37	**ADULT**	**-**	**No**
38	**ADULT**	**-**	**No**
39	**ADULT**	**+ (0.470)**	**Yes**
40	**ADULT**	**-**	**No**
41	**ADULT**	**-**	**No**
42	**ADULT**	**-**	**No**
43	**ADULT**	**-**	**No**
44	**ADULT**	**-**	**No**
45	**ADULT**	**-**	**No**
46	**ADULT**	**-**	**No**
47	**ADULT**	**-**	**No**
48	**ADULT**	**-**	**No**

Out of 48 fecal samples, 11 are found to be rotavirus positive (both by ELISA and VP6 PCR). That accounts for **22.9%** of rotavirus cases. Also it can be seen that rotavirus associated diarrheal cases are more prominent in children (33.33%) as compared to adults (5.5%). More sample size would give a better picture and help in monitoring the rotavirus associated diarrheal cases. Genotyping is an important aspect that needs to be given attention to avoid vaccine pressure (or emergence of vaccine resistant strains). Government of India has introduced vaccine against rotavirus in four of the Indian states as a part of UIP. Still, monitoring would be required continuously as viruses mutate rapidly. Mortality and morbidity linked with rotavirus associated diarrheal cases can be brought down with better monitoring and surveillance networks, vaccination programs, maintaining proper hygiene conditions and spreading awareness among people (especially parents of newborns) about the disease that can be controlled.

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