Abstract:

Title: VACCINE

The invention provides a method of inducing an immune response against rotavirus strain, the method comprising administering to a subject a composition comprising an attenuated rotavirus strain of a GxPy type, said composition generating an immune response against a rotavirus strain which is neither a Gx nor a Py type.
VACCINE

TECHNICAL FIELD

This invention relates to rotavirus vaccine formulations. The invention relates to the use of an attenuated rotavirus population from one rotavirus type in the prevention of disease associated with rotavirus infection from another rotavirus type.

TECHNICAL BACKGROUND

Acute, infectious diarrhoea is a leading cause of disease and death in many areas of the world. In developing countries, the impact of diarrhoeal disease is staggering. For Asia, Africa and Latin America, it has been estimated that there are between 3-4 billion cases of diarrhoea each year and of those cases about 5-10 million result in death (Walsh, J.A. et al.: N. Engl. J. Med., 301:967-974 (1979)).

Rotaviruses have been recognised as one of the most important causes of severe diarrhoea in infants and young children (Estes, M.K. Rotaviruses and Their Replication in Fields Virology, Third Edition, edited by Fields et al., Raven Publishers, Philadelphia, 1996). It is estimated that rotavirus disease is responsible for over one million deaths annually. Rotavirus-induced illness most commonly affects children between 6 and 24 months of age, and the peak prevalence of the disease generally occurs during the cooler months in temperate climates, and year-round in tropical areas. Rotaviruses are typically transmitted from person to person by the faecal-oral route with an incubation period of from about 1 to about 3 days. Unlike infection in the 6-month to 24-month age group, neonates are generally asymptomatic or have only mild disease. In contrast to the severe disease normally encountered in young children, most adults are protected as a result of previous rotavirus infection so most adult infections are mild or asymptomatic (Offit, P.A. et al. Comp. Ther., 8(8):21-26, 1982).

Rotaviruses are generally spherical, and their name is derived from their distinctive outer and inner or double-shelled capsid structure. Typically, the double-shelled capsid structure of a rotavirus surrounds an inner protein shell or core that contains the genome. The genome of a rotavirus is composed of 11 segments of double-stranded
RNA which encode at least 11 distinct viral proteins. Two of these viral proteins designated as VP4 and VP7 are arranged on the exterior of the double-shelled capsid structure. The inner capsid of the rotavirus presents one protein, which is the rotavirus protein designated VP6. The relative importance of these three particular rotaviral proteins in eliciting the immune response that follows rotavirus infection is not yet clear. Nevertheless, the VP6 protein determines the group and subgroup antigen, and VP4 and VP7 proteins are the determinants of serotype (types determined by neutralisation assay) and genotype (types determined by a non-serological assay) specificity. The designations for G serotypes and G genotypes are identical. In contrast, the numbers assigned for P serotypes and genotypes are different (Santos N. et Hoshino Y., 2005, Reviews in Medical Virology, 15, 29-56). Therefore the P serotype is designated as P followed by assigned number, and the P genotype is designated by a P followed by assigned number in brackets.

To date, at least 14 rotavirus G serotypes and 14 rotavirus P serotypes have been identified (Santos N. et Hoshino Y., 2005, Reviews in Medical Virology, 15, 29-56). Among these, 10 G (G1-6, G8-10 and G12) serotypes and 9 P (P1, P2A, P3, P4, P5A, P7, P8, P11 and P12) serotypes have been identified among the human rotavirus. Twenty-three P genotypes have been described ten of which have been recovered from humans (P[3]-[6], P[8]-[11], P[14] and P[19]).

VP7 protein is a 38,000 MW glycoprotein (34,000 MW when non-glycosylated) which is the translational product of genomic segment 7, 8 or 9, depending on the strain. This protein stimulates formation of the neutralising antibody following rotavirus infection. VP4 protein is a non-glycosylated protein of approximately 88,000 MW which is the translational product of genomic segment 4. This protein also stimulates neutralising antibody following rotavirus infection.

Since VP4 and VP7 proteins are the viral proteins against which neutralising antibodies are directed, they are believed to be prime candidates for development of rotavirus vaccines, affording protection against rotavirus illness.

Natural rotavirus infection during early childhood is known to elicit protective immunity.
A live attenuated rotavirus vaccine is thus highly desirable. Suitably this should be an oral vaccine, as this is the natural route of infection of the virus.

Early vaccine development for preventing rotavirus infections began in the 1970s after the discovery of the virus. Initially, attenuated strains from animals and humans were studied and had mixed or disappointing results. More recent efforts have focused on human-animal reassortants that have been more successful.

A rotavirus strain known as 89-12 has been described by Ward; see US Patent Number 5,474,773 and Bernstein, D.L. et al, Vaccine, 16 (4), 381-387, 1998. The 89-12 strain was isolated from a stool specimen collected from a 14 month-old child with natural rotavirus illness in 1988. According to US Patent Number 5,474,773 the HRV 89-12 human rotavirus was then culture-adapted by 2 passages in primary African Green Monkey Kidney (AGMK) cells and 4 passages in MA-104 cells as described by Ward in J. Clin. Microbiol., 19, 748-753, 1984. It was then plaque purified 3 times in MA-104 cells (to passage 9) and grown after 2 additional passages in these cells. One additional passage was made (passage 12) for deposition with the ATCC under the accession number ATCC VR 2272. The deposited strain is known as 89-12C2.

The 1998 paper in Vaccine by Bernstein et al is referred to below as the Vaccine (1998) paper. The paper describes the safety and immunogenicity of an orally administered live human rotavirus vaccine candidate. This vaccine was obtained from strain 89-12, attenuated by passaging without plaque purification 26 times in primary AGMK cells and then another 7 times in an established AGMK cell line (33 passages in total).

Hereinafter the aforesaid material which has been serially passaged 26 times will be referred to as P26 and the material which has been serially passaged 33 times will be referred to as P33. In general, rotavirus derived by passaging 89-12 n times will be referred to as Pn.

In the examples which follow the P33 material was passaged a further 5 times on Vero cells. This is referred to as P38.
The P26 and P33 isolates described in the Vaccine (1998) paper were not deposited in a culture collection, nor were they analysed to establish their genetic characterisation.

It has now been found that the P26 population described in the literature comprises a mixture of variants. This has been established by genetic characterisation as described hereinbelow (see examples). P26 is therefore not a reliably consistent population for further passages, in particular for the production of vaccine lots. Similarly, P33 comprises a mixture of variants and is not reliably consistent for the production of vaccine lots.

It has been found that the P26 material is a mixture of at least three VP4 gene variants. P33 and P38 are similarly a mixture of two variants. These variants appear to be antigenically different, in terms of neutralising epitopes, to the 89-12C2 strain deposited at the ATCC when evaluating the neutralizing antibody titers of sera from infants vaccinated with P33 against these variants.

Furthermore it has been found that when the P33 material is administered to infants, two identified variants are replicated and excreted. Of 100 vaccinated infants, only 2 showed signs of gastro-enteritis due to rotavirus infection, while 20% of a placebo group were infected. These findings suggest that the identified variants are associated with protection from rotavirus disease.

WO 01/12797 discloses a method of separating rotavirus variants and an improved live attenuated rotavirus vaccine derived from a cloned (homogeneous) human rotavirus strain. Also disclosed is an attenuated rotavirus population (isolate), characterised in that it comprises a single variant or substantially a single variant, said variant defined by the nucleotide sequence encoding at least one of the major viral proteins designated as VP4 and VP7. Protective efficacy of such an oral attenuated human rotavirus vaccine against G9 heterologous strain has been reported in Latin American infants (Perez et al. 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2002) 27-30 September 2002, San Diego). WO 05/021033 discloses that one rotavirus serotype may be used to protect against disease caused by another serotype. In particular WO 05/021033 discloses the use of a G1 rotavirus population, [for example as deposited at the European Collection of Animal Cell Cultures (ECACC), Vaccine Research and
Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 OJG, United Kingdom on 13 August 1999 under the deposition number 99081301, under the terms of the Budapest Treaty, also named P43 or RIX4414], to prevent disease caused by both G1 and at least one non-G1 rotavirus serotype, such as but not limited to the G2, G3, G4 and G9 rotavirus serotypes.

The whole content of WO 01/12797 and WO 05/021033 is herein incorporated by reference.

**BRIEF DESCRIPTION OF THE FIGURES**

**Figure 1A** (SEQ ID NO:1) is the nucleotide sequence of P43 (RIX4414) VP4 gene including the sequence encoding the VP4 protein of P43.

**Figure 1B** (SEQ ID NO:2) has additional nucleotides from both ends of the gene and a nucleotide substitution (in bold - a G instead of a C at position 18 with resulting in TCG instead of TCA with however no impact on the resulting encoded protein) due to the sequencing technique. The non-coding sequence appears in small case. Figure 1B shows the correct sequence for the P43 deposit.

**Figure 2A** (SEQ ID NO:3) is the nucleotide sequence of P43 (RIX4414) VP7 gene including the sequence encoding the VP7 protein of P43.

**Figure 2B** (SEQ ID NO:4) has additional nucleotides from both ends of the gene and a nucleotide substitution (in bold - a A instead of a C at position 58, resulting in a ATT coding for leucine instead of CTT coding for isoleucine) due to the sequencing technique. The non-coding sequence appears in small case. Figure 2B shows the correct sequence for the P43 deposit.

**Figure 3** (SEQ ID NO:5) is the polypeptide sequence of RIX4414 VP4.

**Figure 4** (SEQ ID NO:6) is the polypeptide sequence of RIX4414 VP7.

**Figure 5** (SEQ ID NO:7) shows the polypeptide sequence of NSP4 protein of RIX4414.

**Figure 6** (SEQ ID NO:8) shows the nucleotide sequence encoding NSP4 protein of RIX4414. The non-coding sequence appears in small case.

**Figure 7** (SEQ ID NO:9) shows the polypeptide sequence of VP6 protein of RIX4414.

**Figure 8** (SEQ ID NO:10) shows the nucleotide sequence encoding VP6 protein of RIX4414. The non-coding sequence appears in small case.
DETAILED DESCRIPTION OF THE INVENTION

In the present invention we have determined that an attenuated rotavirus population, for example one such as characterised in WO 01/12797, can be used as a vaccine to provide cross protection against disease caused by rotavirus infection of a different type (serotype and/or genotype) to that used in the vaccine. The VP7 protein specifies the G type (serotype), and the VP4 protein specifies the P type of strain (serotype or genotype).

In particular the present invention relates to the use of an attenuated rotavirus population from one P type in the prevention of disease associated with rotavirus infection from a different P type, and specifically to the use of an attenuated rotavirus population or strain from a GxPy type in the induction of an immune response and/or in the prevention of disease associated with rotavirus infection caused by a rotavirus strain which is neither a Gx nor a Py type.

Immunity may be measured by neutralising antibody responses to the vaccine or by serum rotavirus IgA antibody response, such as seroconversion factor (i.e. >3-fold increase in serum antibody IgA levels following vaccination, as described in Ward et al., 1990, J. Infect. Disease, 161, 440-445).

In the context of this invention, and consistent with the common understanding in the art (Santos N. et Hoshino Y., 2005, Reviews in Medical Virology, 15, 29-56), Gx will refer to a specific G type, i.e. G genotype or G serotype (both terminologies being identical), whilst Py terminology will generically refer to a specific P type, either P serotype (e.g. P8, P4) or P genotype (e.g. P[4], P[8]). When referred to a specific P genotype, the P followed by assigned number in brackets will be used; otherwise P type will mean either serotype or genotype.

Throughout this specification, wording such as the use of a vaccine composition according to the invention in the manufacture of a vaccine composition for the prevention of rotavirus diseases, or such as methods of therapy comprising the use of said vaccine composition will be interchangeably used.
We have now determined that a GxP[8] rotavirus population [for example G1P[8] as
deposited at the European Collection of Animal Cell Cultures (ECACC), Vaccine
Research and Production Laboratory, Public Health Laboratory Service, Centre for
Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 OJG, United
Kingdom on 13 August 1999 under the deposition number 99081301, under the terms of
the Budapest Treaty], can be used to prevent disease caused by both a GxP[8] (e.g.
G1P[8]) and at least one rotavirus strain which is neither a Gx nor a Py type. In particular
we have determined that a G1P[8] rotavirus population can be used to prevent disease
caused by both one G1P[8] and at least one non- G1P[8] genotypes, such as G2P[4]
rotavirus genotype.

Accordingly the present invention relates to use of an attenuated rotavirus population
from one rotavirus type in the prevention of disease associated with rotavirus infection
from another rotavirus type, wherein the type is suitably defined by reference to the
sequence of the rotavirus VP4 protein (P type).

The invention also relates to the use of an attenuated rotavirus population from one
rotavirus strain (defined by both a specific G and P type) in the prevention of disease
associated with rotavirus infection from another rotavirus strain, wherein the strain is
suitably defined by reference to the sequence of both the rotavirus VP4 protein (P type)
and VP7 protein (G type). Specifically the present invention relates to the use of an
attenuated rotavirus strain from a GxPy type in the manufacture of a medicament for
inducing an immune response against rotavirus infection caused by a rotavirus strain
which is neither a Gx nor a Py type. In other words, a rotavirus strain of the invention
can be used to prevent disease caused by infection of a second rotavirus which differs in
both the G and P type.

In particular, in all aspects of the claimed invention said immune response is a protective
immune response. Suitably the rotavirus population comprises VP4 and/or VP7 viral
proteins from ECACC deposit 99081301 suitable to provide a cross protective effect.

Throughout the document, it will be referred to cross-protection as being the protection
afforded by a rotavirus type against infection caused by a rotavirus of a different type.
Cross-protection can be homotypic or heterotypic. Homotypic cross-protection is a protection afforded by a rotavirus strain against a strain of either a G or a P type, such as for example a G1P[8] strain affording cross-protection against a non-G1, P[8] strain (e.g. G2P[8]) via the P[8] type. Another example of a homotypic cross-protection is that afforded by a G1P[8] strain against a G1 non-P[8] strain (e.g. G1P[4]) via the G1 type.

Heterotypic cross-protection is a protection afforded by a rotavirus strain against a rotavirus strain of different P and G types such as for example the protection afforded by a G1P[8] against a non G1- non P[8]- strain (e.g. G2P[4]) (heterotypic protection afforded via both G and P types).

Suitably the attenuated rotavirus serotype is G1 and is able to provide cross protection against disease caused by G1 and non-G1 rotavirus serotypes such as serotypes selected from the group consisting of: G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 and G14.

In particular the use of a G1 attenuated rotavirus population, [for example as deposited at the European Collection of Animal Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 0JG, United Kingdom on 13 August 1999 under the deposition number 99081301, under the terms of the Budapest Treaty], can be used to prevent disease caused by G1 and at least one, suitably at least two, suitably at least three, suitably at least four non-G1 rotavirus serotypes selected from the group consisting of: G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 and G14. Accordingly, there is provided the use of an attenuated rotavirus strain from a G1 type in the manufacture of a vaccine composition for the induction of an immune response against a rotavirus infection caused by a rotavirus strain which is not from a G1 type. In a particular aspect, an immune response is induced against at least one, at least two or more rotavirus non-G1 serotypes, typically against any serotype selected from the group consisting of: G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 and G14. Typically an immune response is induced against at least one, suitably at least two, suitably at least three of the following non-G1 types: G2, G3, G4 and G9, in addition to homotypic (G1) protection. Suitably the composition comprises a G1 rotavirus strain and is used to induce an immune response to the G1 and G2 types.
Suitably the rotavirus attenuated strain type is P[8] and is able to provide cross-protection against disease caused by P[8] rotavirus type and by non-P[8] rotavirus types such as types selected from the group consisting of: P[1], P[2], P[3], P[4], P[5], P[6], P[7], P[9], P[10], P[11], P[12], P[14] and P[19].

In particular the use of a P[8] attenuated rotavirus population, [for example as deposited at the European Collection of Animal Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 OJG, United Kingdom on 13 August 1999 under the deposition number 99081301], under the terms of the Budapest Treaty], can be used to prevent disease caused by P[8] and at least one of the non-P[8] rotavirus types, selected from the group consisting of: P[1], P[2], P[3], P[4], P[5], P[6], P[7], P[9], P[10], P[11], P[12], P[14] and P[19]. In particular an immune response is suitably induced against at least a P[4] type in addition to the P[8] rotavirus type.

Suitably the vaccine composition for use according to the invention comprises a G1P[8] rotavirus strain and is capable of inducing an immune response to a G2P[4] rotavirus strain.

In a particular aspect, the invention relates to a method of inducing an immune response against rotavirus strain, the method comprising administering to a subject a composition comprising an attenuated rotavirus strain of a GxPy type, said composition generating an immune response against a rotavirus strain which is neither a Gx nor a Py type.

In particular, the invention relates to a method of inducing an immune response against rotavirus G1 and non-G1 serotype, the method comprising administering to a subject a composition comprising a rotavirus G1 serotype vaccine. Suitably non-G1 rotavirus serotypes are selected from the group consisting of: G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 and G14. Suitably the composition comprises a G1 rotavirus strain and is used to induce an immune response to the G1 and G2 types.
Suitably the vaccine composition for use according to the invention comprises a G1P[8] rotavirus strain and is capable of inducing an immune response to a G2P[4] rotavirus strain.

Suitably the rotavirus population within the vaccine composition is of G1P1A (i.e. G1P[8] according to the current nomenclature) strain specificity. Suitably the rotavirus population comprises VP4 and/or VP7 viral proteins from ECACC deposit 99081301 suitable to elicit an immune response and, typically, provide a cross protective effect. Suitably the invention relates to G1P[8] rotavirus strains in methods or uses as described above. Typically the rotavirus vaccine used is the ECACC deposit 99081301, or is derived from that deposit.

In a specific aspect the vaccine induces a cross-protective immune response or cross-protection against gastro-enteritis in a vaccinated individual compared to the unvaccinated individual (from the placebo group). Suitably the vaccine provides cross-protection against rotavirus infection symptoms such as diarrhea or gastro-enteritis. For example gastro-enteritis may be defined as diarrhea characterised by three or more, watery or looser than normal, stools within a day, or forceful vomiting along with the detection of rotavirus in the examined stool specimen.

As will be understood by the skilled artisan, disease severity and efficacy of the vaccination to induce a protective immune response in a vaccinated individual or a vaccinated population may be assessed by several means. By protective immune response is meant an immune response which leads to a reduction of the severity of clinical symptoms associated with rotavirus infection or that leads to reduced susceptibility to rotavirus infection. Disease severity in an unvaccinated or a vaccinated individual may be graded according to published scoring systems such as the 20-point Vesikari scale or a slightly amended version of said method (Ruuska T et al. Scand. J. Infect. Dis. 1990, 22, 259-267), or according to any other suitable system reporting and grading specific symptoms of the rotavirus infection (such as the methodoly reported in Clark HF, Borian EF, Bell LM. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. J Infect Dis. 1988:570-86). According to the Vesikari method, severe RVGE is usually defined as a score ≥11.
Protection may be assessed at the level of a population or a group by vaccine efficacy (VE). Vaccine efficacy is calculated using the following formula:

\[ \text{VE} \% = 1 - \frac{\text{RR}}{1} = 1 - \left( \frac{\text{ARV}}{\text{ARU}} \right) \]

where:
- \( \text{RR} \) = relative risk = \( \frac{\text{ARV}}{\text{ARU}} \)
- \( \text{ARU} \) = disease attack rate in unvaccinated population (estimated from the placebo group) = number of subjects reporting at least one RV GE episode / total number of subjects in the control group.
- \( \text{ARV} \) = disease attack rate in vaccinated group = number of subjects reporting at least one RV GE episode / total number of subjects in the HRV vaccine group.

Accordingly, in one aspect of the present invention, there is provided a method or use as detailed above wherein the composition comprising an attenuated rotavirus strain of a GxPy type induces a cross-protective immune response and/or protection against rotavirus-induced gastro-enteritis, suitably against severe rotavirus-induced gastroenteritis, caused by infection of a rotavirus strain which is neither a Gx nor a Py type. In a specific embodiment said protective immune response is capable of reducing the severity of the disease or eliminate rotavirus induced disease as measured according to any suitable scoring system.

In still another embodiment, there is provided a method or use of the composition according to the invention, to reduce the severity of the disease, e.g. gastroenteritis, or to eliminate rotavirus induced disease, said disease severity or disease being recorded according to any suitable scoring system as taught above.

In a specific embodiment, said composition is up to 60% protective, suitably up to 81% protective, in a population of vaccinated individuals, against diarrhoea caused by infection of a rotavirus of a different type to that of the attenuated rotavirus present in the composition. In another specific embodiment, said composition is at least 40% protective, suitably at least 45% protective, suitably at least 50% protective, suitably at least 60% protective, in a population of vaccinated individuals, against diarrhoea caused by a rotavirus strain which is neither a Gx nor a Py type. In a specific aspect said composition is between 40% and 80% protective, suitably between 50% and 70% protective against diarrhoea caused by a rotavirus strain which is neither a Gx nor a Py
type. In a specific aspect, said composition comprises a G1P[8] rotavirus strain which affords the level of protection as mentioned above against gastro-enteritis caused by infection of rotavirus strains of G2P[4] type.

Suitably the protection rate against diarrhoea and/or gastro-enteritis and/or severe gastro-enteritis achieved in a population of vaccinated individuals infected by a rotavirus strain which is neither a Gx nor a Py type, is between 10 to 90%, suitably between 20 to 80%, suitably between 40% and 80%, suitably between 45% and 75% protective. Typically the level of protection against severe gastro-enteritis is at least 40%, suitably at least 50%.

In a specific aspect, said composition comprises a G1P[8] rotavirus strain which is between 40% and 80% protective, suitably between 45% and 75% protective, in a population of vaccinated individuals against severe gastro-enteritis, as measured according to the Vesikari score, caused by infection of rotaviruses of with a G2P[4] serotype.

Suitably the vaccine is used in a 2 dose or a 3 dose regime.

The rotavirus vaccine used to give cross protection has the following suitable features.

In one aspect, the rotavirus of the composition for use according to the invention has a VP4 gene comprising a nucleotide sequence comprising at least one of the following: an adenine base (A) at position 788, an adenine base (A) at position 802 and a thymine base (T) at position 501 from the start codon.

In a further aspect the rotavirus of the composition for use according to the invention has a VP7 gene comprising a nucleotide sequence comprising at least one of the following: a thymine (T) at position 605, an adenine (A) at position 897, or a guanine (G) at position 897 from the start codon. Suitably at position 897 there is an adenine (A).

In a specific aspect the rotavirus of the composition for use according to the invention has an adenine (A) at positions 788 and 802 and a thymine (T) at position 501 from the start codon in the VP4 gene sequence.
In another specific aspect the rotavirus of the composition for use according to the invention has a thymine (T) at position 605 and an adenine/guanine (A/G) at position 897 from the start codon in the VP7 sequence. Most suitably in the VP7 sequence there is an adenine (A) at position 897.

In a particularly suitable aspect the rotavirus of the composition for use according to the invention has an adenine (A) at positions 788 and 802 and a thymine (T) at position 501 from the start codon in the VP4 gene sequence, and a thymine (T) at position 605 and an adenine/guanine (A/G) at position 897 from the start codon in the VP7 sequence. Suitably in the VP7 sequence there is an adenine (A) at position 897.

In another aspect the rotavirus of the composition for use according to the invention comprises a nucleotide sequence encoding a VP4 protein wherein the nucleotide sequence is as shown in Figure 1A (SEQ ID NO:1) or Figure 1B (SEQ ID NO:2), and/or a nucleotide sequence encoding a VP7 protein wherein the nucleotide sequence is as shown in Figure 2A (SEQ ID NO:3) or Figure 2B (SEQ ID NO:4). In an alternative embodiment the rotavirus of the composition for use according to the invention comprises a VP4 protein as set forth is Figure 3 (SEQ ID NO:5), and/or a VP7 protein as set forth is Figure 4 (SEQ ID NO:6). In another embodiment, said rotavirus population for use according to the invention additionally comprises an NSP4 protein as set forth in Figure 5 (SEQ ID NO:7), or encoded by the nucleotide sequence as set forth in Figure 6 (SEQ ID NO:8), and/or a VP6 protein as set forth in Figure 7 (SEQ ID NO:9), or encoded by the nucleotide sequence as set forth in Figure 8 (SEQ ID NO:10).

Suitable rotavirus populations for use in the present invention may be obtained by a method comprising: passaging a rotavirus preparation on a suitable cell type; optionally selecting homogeneous culture using the steps of either:

a) limit dilution; or

b) Individual plaque isolation; and

checking for the presence of a substantially single variant by carrying out a sequence determination of an appropriate region of the VP4 and/or VP7 gene sequence.
Suitably the rotavirus population is derived from the P43 (RIX4414), P33 or P26 strains as described above.

The sequence determination may suitably be carried out by a quantitative or semi-quantitative hybridisation technique such as slot blot hybridisation or plaque hybridisation.

The resulting cloned virus population resulting from the method according to the invention may be amplified by further passaging on a suitable cell line.

Suitable cell types for passaging the rotavirus population in the above method include African green monkey kidney (AGMK) cells, which may be established cell lines or primary AGMK cells. Suitable AGMK cell lines include for example Vero (ATCC CCL-81), DBS-FRhL-2 (ATCC CL-160), BSC-1 (ECACC 8501 1422) and CV-1 (ATCC CCL-70). Also suitable are MA-104 (rhesus monkey) and MRC-5 (human -ATCC CCL-171) cell lines. Vero cells are particularly Suitable for amplification purposes. Passaging on Vero cells gives a high virus yield.

Techniques for checking whether there is a single variant in a virus population resulting from the method, and for determining the nature of that single variant involve standard sequencing or hybridisation procedures known in the art and are described hereinbelow.

In a specific aspect the method of the invention is carried out using an appropriate rotavirus, particularly rotavirus having the characteristics of the 89-12 strain or of a passaged derivative thereof.

A particularly suitable single variant population is P43, which was obtained from P33 (an isolated human rotavirus passages 33 times in culture on appropriate cell types) by a series of end dilution cloning steps followed by passaging the cloned material on Vero cells for amplification.

A P43 population was deposited at the European Collection of Animal Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury,
Wiltshire, SP4 OJG, United Kingdom on 13 August 1999 under the deposition number 99081301, under the terms of the Budapest Treaty, and is disclosed in WO 01/12797.

Although this indicated public availability is the simplest method of obtaining the human rotavirus P43, similar and functionally substantially identical rotaviruses may be produced by these or other methods in view of the teachings of this invention. Such functionally substantially identical rotaviruses are considered to be biologically equivalent to the human rotavirus P43 of this invention and therefore are within the general scope of the present invention. It will therefore be understood that the invention encompasses rotavirus populations having the characteristics of the P43 variant as described herein.

It will also be understood that the invention encompasses materials derived from the deposited P43 ECACC 99081301 by subjecting it to further processing such as by propagating it by further passaging, cloning, or other procedures using the live virus or by modifying P43 in any way including by genetic engineering techniques or reassortant techniques. Such steps and techniques are well known in the art.

Materials derived from the deposited P43 which are covered by the invention include protein and genetic material. Of particular interest are reassortant rotaviruses which comprise at least one antigen or at least one segment of P43, for example reassortants which comprise a virulent strain of rotavirus in which one or part of one of the 11 genome segments has been replaced by the genome segment or part thereof of P43. Specifically, a rotavirus reassortant in which the segment or partial segment coding for NSP4 is a P43 segment or partial segment, may have useful properties. Reassortant rotaviruses and techniques for preparing them are well known (Foster, R. H. and Wagstaff, A. J. Tetravalent Rotavirus Vaccine, a review. ADIS drug evaluation, BioDrugs, Gev, 9 (2), 155-178, 1998).

Materials of particular interest are progeny of P43 and immunologically active derivatives of P43. Immunologically active derivatives means materials obtained from or with the P43 virus, particularly antigens of the virus, which are capable of eliciting an immune response that is reactive against Rotavirus when injected into a host animal.
In adapting the rotavirus to an appropriate cell line, for example Vero cells, it may be
necessary to treat the virus so as to get rid of any potential contaminant such as any
adventitious agents that may be present and which would otherwise cause contamination. In the case of ether-sensitive adventitious viruses, this may be done by
ether treatment as described hereinbelow. The present invention also relates to
inclusion of such ether treatment as an optional step in the overall procedure for
obtaining an attenuated live rotavirus or vaccine formulated therewith.

The cross protective rotavirus strain of the present invention may be combined with other
rotavirus strains to provide additional protection or cross-protection against rotavirus
infection or disease.

The present invention also provides a live attenuated rotavirus vaccine capable of
providing cross protection, as defined herein above, admixed with a suitable adjuvant or
a pharmaceutical carrier.

In one embodiment, the rotavirus vaccine for use according to the invention is a
monovalent rotavirus vaccine containing a single rotavirus strain such as the G1P[8]
strain.

The present invention is particularly advantageous in providing a live rotavirus vaccine in
which the live attenuated rotavirus is a human rotavirus and does not cause
intussusception.

Suitable pharmaceutical carriers for use with the attenuated rotavirus strain according to
the invention include those known in the art as being suitable for oral administration,
especially to infants. Such carriers include and are not limited to carbohydrates,
polyalcohols, amino acids, aluminium hydroxide, magnesium hydroxide, hydroxyapatite,
talc, titanium oxide, iron hydroxide, magnesm stearate, carboxymethylcellulose,
hydroxypropylmethylcellulose, microcrystalline cellulose, gelatin, vegetal peptone,
xanthane, caraghenane, arabic gum, β-cyclodextrin.
The invention also provides a process for preparing a rotavirus vaccine, for example by freeze drying the virus in the presence of suitable stabilisers or admixing the virus according to the invention with a suitable adjuvant or pharmaceutical carrier.

It may also be advantageous to formulate the virus of the invention in lipid-based vehicles such as virosomes or liposomes, in oil in water emulsions or with carrier particles. Alternatively or in addition immunostimulants such as those known in the art for oral vaccines may be included in the formulation. Such immunostimulants include bacterial toxins, particularly cholera toxin (CT) in the form of the holotoxin (entire molecule) or the B chain only (CTB) and the heat labile enterotoxin of *E. coli* (LT).

Mutated LTs (mLTs) which are less likely to convert to their active form than the native LT are described in WO 96/06627, WO 93/13202 and US 5,182,109.

Further immunostimulants which may advantageously be included are saponin derivatives such as QS21 and monophosphoryl lipid A, in particular 3-de-O-acylated monophosphoryl lipid A (3D-MPL). Purified saponins as oral adjuvants are described in WO 98/56415. Saponins and monophosphoryl lipid A may be employed separately or in combination (e.g. WO 94/00153) and may be formulated in adjuvant systems together with other agents. 3D-MPL is a well-known adjuvant manufactured by Ribi Immunochem, Montana and its manufacture is described in GB 2122204.


The invention also provides a method for vaccinating human subjects, especially infants, by administering to a subject in need thereof an effective amount of a vaccine composition according to the invention. Suitably the live attenuated vaccine is administered by oral administration.

In a specific aspect the attenuated rotavirus strain according to the invention is formulated with an antacid to minimise inactivation of the vaccine by acid in the stomach. Suitable antacid components include inorganic antacids for example aluminium hydroxide Al(OH)_3 and magnesium hydroxide Mg(OH)_2. Commercially available
antacids which are suitable for use in the invention include Mylanta (trade mark) which contains aluminium hydroxide and magnesium hydroxide. These are insoluble in water and are given in suspension.

Aluminium hydroxide is a particularly suitable component of a vaccine composition according to the invention as it can provide not only an antacid effect but also an adjuvantation effect.

Also suitable for use as antacids in the vaccine of the invention are organic antacids such as organic acid carboxylate salts. A suitable antacid in the vaccine composition of the invention contains an organic acid carboxylate salt, specifically a salt of citric acid such as sodium citrate or potassium citrate.

A particularly suitable antacid that may be used in the vaccine composition of the present invention is the insoluble inorganic salt, calcium carbonate (CaCO₃). The calcium carbonate is able to associate with the rotavirus and the rotavirus activity is maintained during the association with the calcium carbonate.

To prevent sedimentation of calcium carbonate during the filling step, viscous agents are suitably present in the formulation.

Possible viscous agents that may be used include pseudoplastic excipients. A pseudoplastic solution is defined as a solution having higher viscosity on standing compared to its viscosity under agitation. Excipients of this type are natural polymers such as arabic gum, adragante gum, agar-agar, alginates, pectines or semi-synthetic polymers for example: carboxymethylcellulose (Tyloses C®), methylcellulose (Methocels A®, Viscotrans MC®, Tylose MH® and MB®), hydroxypropylcellulose (Klucels®), and hydroxypropylmethylcellulose (Methocels E® and K®, Viscontrans MPHC®). In general those pseudoplastic excipients are used together with thixotropic agents. Alternative viscous agents that may be used are pseudoplastic excipients with low flowing capacity. Those polymers, at a sufficient concentration, give rise to a structural fluid arrangement resulting in a high viscosity solution having low flowing capacity on standing. A certain quantity of energy needs to be given to the system to allow flowing and transfer.
External energies (agitation) are needed to destroy temporarily the structural fluid arrangement in order to obtain a fluid solution. Examples of such polymers are Carbopols® and xanthane gum.

Thixotropic excipients become a gel structure on standing whilst under agitation they form a fluid solution. Examples of thixotropic excipients are: Veegum ©(Magnesium-aluminium silicate) and Avicel RC® (about 89% microcrystalline cellulose and 11% Carboxymethylcellulose Na).

The vaccine composition of the present invention suitably comprises a viscous agent selected from xanthane gum or starch.

Thus the vaccine composition of the present invention is typically formulated with a combination of calcium carbonate and xanthane gum.

Other components of a composition used in the invention suitably include sugars for example sucrose and/or lactose.

The vaccine composition according to the invention may contain additional components including for example flavourings (particularly for an oral vaccine) and bacteriostatic agents.

Different presentations of the vaccine composition according to the invention are envisaged.

In one suitable embodiment, the vaccine is administered as a liquid formulation. Suitably the liquid formulation is reconstituted prior to administration from at least the following two components:

i) virus component

ii) liquid component.

In this embodiment, the virus component and the liquid component are normally present in separate containers, which may conveniently be separate compartments of a single
vessel, or separate vessels which can be connected in such a way that the final vaccine composition is reconstituted without exposing it to the air.

Prior to reconstitution, the virus may be in a dry form or a liquid form. Suitably the virus component is lyophilised. Lyophilised virus is more stable than virus in an aqueous solution. The lyophilised virus may be suitably reconstituted using a liquid antacid composition to produce a liquid vaccine formulation. Alternatively the lyophilised virus may be reconstituted with water or aqueous solution, in which case the lyophilised virus composition suitably contains an antacid component.

Suitably, the vaccine formulation comprises a virus component formulated with calcium carbonate and xanthane gum in one compartment or vessel and this is reconstituted with water or aqueous solution present in the second compartment or vessel.

In another embodiment, the vaccine composition is a solid formulation, suitably a lyophilised cake which is suitable for immediate dissolution when placed in the mouth. Lyophilised formulations may conveniently be provided in the form of tablets in a pharmaceutical blister pack.

In another aspect the invention provides a rotavirus vaccine in the form of a quick dissolving tablet for oral administration.

In another aspect the invention provides a composition comprising a live attenuated rotavirus strain, in particular a human rotavirus strain, wherein the composition is a lyophilised solid capable of immediate dissolution when placed in the mouth.

Suitably the quick dissolving tablet according to the invention dissolves in the mouth of the subject sufficiently quickly to prevent swallowing of the undissolved tablet. This approach is particularly advantageous for paediatric rotavirus vaccines.

Suitably the virus is a live attenuated human rotavirus which is formulated with an inorganic antacid such as calcium carbonate and a viscous agent such as xanthane gum.
A further aspect of the present invention is to provide a lyophilised formulation wherein the virus component is any rotavirus strain which is formulated with calcium carbonate and xanthane gum.

Vaccines of the invention may be formulated and administered by known techniques, using a suitable amount of live virus to provide effective protection against rotavirus infection without significant adverse side effects in typical vaccinees. A suitable amount of live virus will normally be between $10^4$ and $10^7$ focus forming units (ffu) per dose. A typical dose of vaccine may comprise $10^5$—$10^6$ffu per dose and may be given in several doses over a period of time, for example in two doses given with a two-month interval. Benefits may however be obtained by having more than 2 doses, for example a 3 or 4 dose regimen, particularly in developing countries. The interval between doses may be more or less than two months long. An optimal amount of live virus for a single dose or for a multiple dose regimen, and optimal timing for the doses, can be ascertained by standard studies involving observation of antibody titres and other responses in subjects.

The vaccine of the invention may also comprise other suitable live viruses for protection against other diseases, for example poliovirus. Alternatively other suitable live virus vaccines for oral administration may be given in a separate dose but on the same occasion as the rotavirus vaccine composition according to the invention.

Sera from twelve 4 to 6 month old infants vaccinated with the P33 material as described in the Vaccine (1998) paper were tested for neutralization of P33, P38, P43 and 89-12C2.

The range of neutralization titers of all the tested sera is similar for P33, P38 and P43. The statistical analysis shows no significant difference in the overall neutralization titers against all three viruses. This suggests that the conformational and non-conformational neutralization epitopes of P33, P38 and P43 are equally well recognized by the anti-P33 sera of P33 vaccinated infants. This observation indirectly suggests that the neutralization epitopes revealed in this in vitro assay were not altered between P33, P38 and P43.
The range of neutralization titers of P89-1 2C2 however significantly differs from P33, P38 and P43. This observation suggests that the conformational and non-conformational neutralization epitopes of P33, P38 and P43 are not equally well recognized by the anti-P33 sera of P33 vaccinated infants. This observation indirectly suggests that the neutralization epitopes revealed in this in vitro assay were altered between 89-12 C2 and P33, P38 and P43.

Particularly suitable embodiments of the present invention include:

1. The use of an attenuated rotavirus strain from a P type in the manufacture of a vaccine composition for the induction of an immune response against a rotavirus of a different P type to that of said vaccine composition.

2. The use of an attenuated rotavirus strain from a P[8] type in the manufacture of a vaccine composition for the induction of an immune response against a rotavirus which is not P[8].

3. The use of an attenuated rotavirus strain from a G1P[8] type in the manufacture of a vaccine composition for the induction of an immune response against a rotavirus which is not G1P[8].

4. The use according to 1 to 3 wherein an immune response is additionally induced against rotavirus infection by a G1P[8] type.

5. The use according to 1 to 4 wherein the immune response is induced against two or more rotavirus serotypes, these serotypes being defined by reference to the G or P types.

6. The use according to 1 to 5 wherein the serotype of the vaccine strain is a G1 serotype and the non-G1 serotype is selected from the list consisting of: G2, G3, G4, G5, G6, G7, G8, G10, G11, G12, G13 and G14.

7. The use according to 6 wherein an immune response is induced against both the G1 type and the G2 type.
8. The use according to any of 1 to 5 wherein the type of the vaccine strain is a P[8]
type and the non- P[8] type is selected from the list consisting of: P[1], P[2], P[3],

9. The use according to 8 wherein an immune response is induced against both the

10. The use according to any of 1 to 9 wherein the composition comprises a rotavirus
having a VP4 gene comprising, in the nucleotide sequence, at least one of the
following: an adenine base (A) at position 788, an adenine base (A) at position 802
and a thymine base (T) at position 501 from the start codon.

11. The use according to 10 wherein the VP4 gene comprises a nucleotide sequence
comprising an adenine base (A) at positions 788 and 802 and a thymine base (T) at
position 501 from the start codon.

12. The use according to 11 wherein the composition comprises a rotavirus having a
VP7 gene comprising, in the nucleotide sequence, at least one of the following: a
thymine (T) at position 605, an adenine (A) at position 897 and a guanine (G) at
position 897 from the start codon.

13. The use according to 12 wherein the VP7 gene comprises a nucleotide sequence
comprising a thymine (T) at position 605 and an adenine (A) or a guanine (G) at
position 897 from the start codon.

14. The use according to any of 1 to 13, wherein the composition comprises a rotavirus
having a VP4 gene comprising, in the nucleotide sequence, an adenine (A) at
positions 788 and 802 and a thymine (T) at position 501 from the start codon; and
wherein the VP7 gene comprises, in the nucleotide sequence, a thymine (T) at
position 605 and an adenine (A) at position 897 from the start codon.

15. The use according to any of 1 to 14 wherein the composition is capable of reducing
or protecting against gastro-enteritis and/or diarrhea caused by infection by a
rotavirus of a different type defined by reference to either the G and/or the P type of
the attenuated rotavirus present in the composition.

16. The use according to 15 wherein the composition is at least 40% protective in a
population of vaccinated individuals against severe gastro-enteritis caused by
infection of rotavirus of at least two strains defined by reference to either the G
and/or the P type, these types being different to the G1P[8] type of the attenuated
rotavirus present in the composition.

17. The use according to 16 wherein the severe gastro-enteritis is caused by infection of
a rotavirus of at least three, at least four, non-G1 serotypes.

18. The use according to 17 wherein the non-G1 serotypes are any of G2, G3, G4 and
G9 serotypes.

19. The use according to 18 wherein the severe gastro-enteritis is caused by infection of
a rotavirus of at least two non-P[8] types.

20. The use according to 9 wherein the severe gastro-enteritis is caused by infection of a

21. The use according to any of 1 to 20 wherein the rotavirus strain is ECACC deposit
99081 301, or is obtainable or derivable from ECACC deposit 99081 301.

23. The use according to any of 1 to 20 wherein the vaccine is used in a 2-dose regime.

In another aspect, the invention also relates to a method of inducing an immune
response against rotavirus infection from a rotavirus strain, the method comprising
administering to a subject a composition comprising an attenuated rotavirus vaccine
from a different strain. Specifically the invention relates to a method for inducing an
immune response against rotavirus from one P type and/or for preventing disease
associated with rotavirus infection from one P type, said method comprising
administering to a patient in need thereof an attenuated rotavirus population from a
different P type.
In a specific aspect of the invention there is provided a method of inducing an immune response to P[8] rotavirus type and at least one of the non-P[8] types selected from the group consisting of: P[I], P[2], P[3], P[4], P[5], P[6], P[7], P[9], P[11], P[12], P[14] and P[19] types, suitably to the P[4] rotavirus type, the method comprising administering to a subject a composition comprising a rotavirus P[8] type vaccine.

In another aspect of the invention there is provided i) an isolated non-structural protein 4 (NSP4) protein sequence as set forth in Figure 5 (SEQ ID NO:7) or immunogenic fragment thereof; ii) an isolated polynucleotide sequence which comprises a nucleic acid sequence encoding said NSP4 polypeptide, or immunogenic fragment thereof; iii) an isolated polynucleotide sequence which comprises a nucleic acid sequence as set forth in Figure 6 (SEQ ID NO:8).

In still another aspect of the invention there is provided i) an isolated rotavirus protein 6 (VP6) protein sequence as set forth in Figure 7 (SEQ ID NO:9) or immunogenic fragment thereof; ii) an isolated polynucleotide sequence which comprises a nucleic acid sequence encoding said VP6 polypeptide, or immunogenic fragment thereof; iii) an isolated polynucleotide sequence which comprises a nucleic acid sequence as set forth in Figure 8 (SEQ ID NO:10).

Immunogenic fragments may be defined in the context of this invention as fragments that when administered at an effective dose (either alone or as a hapten bound to a carrier) elicit a protective immune response against rotavirus infection.
The following, non-limiting, examples illustrate the invention.

EXAMPLES

EXAMPLE 1: Demonstration that strain 89-12 at passage 26 (P26) is a mixture of variants

Sequencing of VP4 and VP7 genes from different passage lots

Sequencing of VP4 and VP7 genes from passage P26 (primary AGMK cells), passage P33 (established (as opposed to primary) AGMK cell line), passage P41 and passage P43 was performed. Total RNA extraction was reverse transcribed and amplified through PCR in one tube/one step.

Primers Rota 5bis and Rota 29bis amplified the entire VP4 gene and primers Rota 1 and Rota 2bis amplified the entire VP7 gene. The PCR material has been sequenced using different primers (see Table 1).

The passage P26 sequence differed from the passage P33 sequence by 3 bases (at positions 501, 788 and 802 bp from the start codon) in VP4 and by three bases in VP7 (108, 605 and 897 bp from the start codon).

The passage P26 sequence scans of VP4 and VP7 show at mutated positions the presence of the passage P33 sequence as a background. Thus it can be seen that passage P26 is a mixture of at least 2 variants.

The passage P33 sequence scans seem homogenous in VP4 and heterogeneous for VP7 (see Table 2).

Passage P38 (derived from passage 33) was passaged 5 times on Vero cells and displayed the same set of VP4 and VP7 sequences as passage P33 (AGMK cell line). Thus there was no major change in populations between P33 and P38.

TABLE 1: Oligonucleotides used for RT-PCR and sequencing
<table>
<thead>
<tr>
<th>name</th>
<th>sequence</th>
<th>position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rota 1</td>
<td>GGC TTT AAA AGA GAG AAT TTC CGT CTG G (SEQ ID NO:11)</td>
<td>-49 to -22</td>
</tr>
<tr>
<td>Rota 1bis</td>
<td>GGT TAG CTC CTT TTA ATG TAT GGT A (SEQ ID NO:12)</td>
<td>-16 to 10</td>
</tr>
<tr>
<td>Rota 2bis</td>
<td>GGT CAC ATC GAA CAA TTC TAA TCT AAG (SEQ ID NO:13)</td>
<td>1014-988</td>
</tr>
<tr>
<td>Rota 7</td>
<td>CAA GTA CTC AAA CCA ATG AGT G (SEQ ID NO:14)</td>
<td>266-287</td>
</tr>
<tr>
<td>Rota 12</td>
<td>TGT TGA TTT TTC TCT CGA TCC AC (SEQ ID NO:15)</td>
<td>372-394</td>
</tr>
<tr>
<td>Rota 46</td>
<td>GGT TGC TGA GAA TGA GAA ATT AGC TAT AGT GG (SEQ ID NO:16)</td>
<td>651-662</td>
</tr>
<tr>
<td>Rota 18</td>
<td>CCA CTA TAG CTA ATT TCT CAT TCT CAG CAA CC (SEQ ID NO:17)</td>
<td>682-651</td>
</tr>
<tr>
<td>Rota 5</td>
<td>TGG CTT CGC CAT TTT ATA GAC A (SEQ ID NO:18)</td>
<td>721-745</td>
</tr>
<tr>
<td>Rota 6</td>
<td>ATT TCG GAC CAT TTA TAA CC (SEQ ID NO:19)</td>
<td>753-727</td>
</tr>
<tr>
<td>Rota 5bis</td>
<td>TGG CTT CAC TCA TTT ATA GAC A (SEQ ID NO:20)</td>
<td>878-856</td>
</tr>
<tr>
<td>Rota 6bis</td>
<td>ATT TCA GAC CAT TTA TAA CCT AG (SEQ ID NO:21)</td>
<td>2-23</td>
</tr>
<tr>
<td>Rota 25</td>
<td>GGA GTA GTA TAT GAA AGT ACA AAT AG (SEQ ID NO:22)</td>
<td>268-296</td>
</tr>
<tr>
<td>Rota 26</td>
<td>CTA TTA TTT GTA CTT TCA TAT ACT ACT CC (SEQ ID NO:23)</td>
<td>296-268</td>
</tr>
<tr>
<td>Rota 27bis</td>
<td>TCG ATA CAG TAT AGA GGA GCA CAA G (SEQ ID NO:24)</td>
<td>721-745</td>
</tr>
<tr>
<td>Rota 28</td>
<td>TTC ATT AAC TTG TGC TCT TCT ATA CTG (SEQ ID NO:25)</td>
<td>1465-1487</td>
</tr>
<tr>
<td>Rota 31</td>
<td>GTA TAT GTA GAC TAT TGG GAT G (SEQ ID NO:26)</td>
<td>1487-1465</td>
</tr>
<tr>
<td>Rota 32</td>
<td>CAT CCC AAT AGT CTA CAT ATA C (SEQ ID NO:27)</td>
<td>1487-1465</td>
</tr>
<tr>
<td>Rota 45</td>
<td>TGT AAC TCC GGC AAA ATG CAA CG (SEQ ID NO:28)</td>
<td>1703-1727</td>
</tr>
<tr>
<td>Rota 53</td>
<td>CGT TGC ATT TTG CCG GAG TTA CA (SEQ ID NO:29)</td>
<td>1727-1703</td>
</tr>
<tr>
<td>Rota 54</td>
<td>GTA AGA CAA GAT TTA GAG CGC CA (SEQ ID NO:30)</td>
<td>2008-2032</td>
</tr>
<tr>
<td>Rota 55</td>
<td>TGG CGC TCT AAA TCT TGT CTG ACT (SEQ ID NO:31)</td>
<td>2335-2311</td>
</tr>
<tr>
<td>Rota 40</td>
<td>CTT GAT GCT GAT GAA GCA GCA TCT G (SEQ ID NO:32)</td>
<td>2335-2311</td>
</tr>
<tr>
<td>Rota 39</td>
<td>CAG ATG CTG CTT CAT CAG CAT CAA G (SEQ ID NO:33)</td>
<td>2335-2311</td>
</tr>
<tr>
<td>Rota 33</td>
<td>CGA TCA TAT CGA ATA TTA AAG GAT G (SEQ ID NO:34)</td>
<td>2335-2311</td>
</tr>
<tr>
<td>Rota 34</td>
<td>CAT CCT TTA ATA TCC GAT ATG ATC G (SEQ ID NO:35)</td>
<td>2335-2311</td>
</tr>
<tr>
<td>Rota 29bis</td>
<td>AGC GTT CAC ACA ATT TAC ATT GTA G (SEQ ID NO:36)</td>
<td>2335-2311</td>
</tr>
</tbody>
</table>

TABLE 2: oligonucleotides used in hybridization
The bases shown in bold type in Table 2 are the sites of specific sequence variation in VP4 and VP7.

TABLE 3: sequence variation of VP4 and VP7 genes

<table>
<thead>
<tr>
<th></th>
<th>VP4</th>
<th>VP7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P26 (AGMK)</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>P33 (AGMK)</td>
<td>T</td>
<td>G / A</td>
</tr>
<tr>
<td>P38 (VERO)</td>
<td>T</td>
<td>G / A</td>
</tr>
<tr>
<td>P43 (VERO)</td>
<td>T</td>
<td>G / A</td>
</tr>
</tbody>
</table>

N.B. In a second clone from the 3 clones which were developed to the level of production lot, the VP7 897 bp position nucleotide is G, rather than A as in the P43 selected clone. This results in a methionine in place of an isoleucine in the amino acid sequence. Variants corresponding to both the selected P43 clone and the clone in which there is a G in VP7 at 897 bp from the start codon, were excreted in the stools of infants who had been vaccinated with the P33 material.

In Table 3.1, where there are two alternative bases at a particular position, the first of the two represents the base which appears in a major population and the second is the base which appears in a minor population. Major and minor variant populations are judged by the strength of the signal in sequencing.

Table 3.2

<table>
<thead>
<tr>
<th></th>
<th>VP4</th>
<th>VP7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P26 (AGMK)</td>
<td>Leu</td>
<td>Arg</td>
</tr>
<tr>
<td>P33 (AGMK)</td>
<td>Phe</td>
<td>Arg/Arg</td>
</tr>
<tr>
<td>P38 (VERO)</td>
<td>Phe</td>
<td>Arg/Arg</td>
</tr>
<tr>
<td>P43 (VERO)</td>
<td>Phe</td>
<td>Arg/Arg</td>
</tr>
</tbody>
</table>
Table 3.2 shows the amino acid changes resulting from the nucleotide differences between the variants.

### TABLE 4

<table>
<thead>
<tr>
<th></th>
<th>VP4 (788-802 positions)</th>
<th>VP7 (897 position)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P26</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P33</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P38</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>P43</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Slot blot hybridization**

The change in populations between passages P26 to P33 on AGMK cells has been further confirmed by slot blot hybridization. The VP4 and the VP7 gene fragments generated by RT/PCR were hybridized with oligonucleotide probes specific for each variant (see Table 3.1 and 3.2). In contrast to P26 which hybridized with Rota 16, Rota 35 and Rota 36 and not with Rota 15, the VP4 PCR fragment of the P33 material, at positions 788 and 802 hybridized only with Rota 16 and not with either Rota 15 or Rota 35 or Rota 36. These results established the presence of at least 3 variants in P26 (see Table 4).

For the VP7 PCR fragment of the P33 material, position 897 hybridized with Rota 41 and Rota 42. These results established the presence of at least two variants in the P33 material.

**EXAMPLE 2: Isolation and characterization of the P43 clone**

To isolate P33 components as a homogeneous virus population, three end-point dilutions of P33/AGMK on Vero cells were performed and the resulting virus was used to infect Vero cells.

Positive wells were selected using two criteria: growth demonstrated by the largest number of foci detected in the wells and the most isolated positive wells on the plates, as
is done classically. After 3 end dilution passages in 96 well microtiter plates, 10 positive
wells were amplified successively on Vero cells and evaluated for their yield.
Based on yield, three clones were developed to passage level of production lot.
Immunorecognition by polyclonal antibodies was shown to be similar both between the
three clones and between the clones and P33. Homogeneity of the clones was assessed
by slot blot hybridization. The final selection of a single clone was based on yield and
sequence.
The selected clone was amplified by successive passages on Vero cells to generate a
Master seed, a Working seed and finally production lots.
The selected clone was genetically characterized at different passage levels by
sequencing of VP4 and VP7 (identity) and by specific slot blot hybridization of the VP4
and VP7 (homogeneity) of the PCR amplified materials. The sequence of the VP4 and
VP7 genes of the P43 material are given in Figures 1 and 2 respectively and are
identical to P41.
Homogeneity of the selected clone was assessed by a selective hybridization using
oligonucleotide probes discriminating nucleotide changes in VP4 and/or VP7 regions for
each variant identified during sequencing of P26/primary AGMK (see Table 4).
The VP4 fragment hybridized with Rota 16 and not with Rota 15, Rota 35 or Rota 36.
The VP7 fragment hybridized with Rota 41 and not with Rota 42.
These results confirmed that P43 is a homogeneous population.

**EXAMPLE 3: Removal of potential adventitious virus**

Ether was added to P33 (AGMK grown) to a final concentration of 20% for 1 hr. Ether
was then bubbled out with N₂ for 35 min. No impact on the titre of P33 seed was
observed.

**EXAMPLE 4: Formulation of a live attenuated vaccine**

The production lots described above are formulated for oral administration to infants by
the following method.
1. **Lyophilised virus**

Standard techniques are used for preparing virus doses. Frozen purified viral bulk is thawed and diluted with appropriate medium composition, in this case Dulbecco’s modified eagle Medium, up to a desired standard viral concentration, in this case $10^{6.2}$ ffu/ml. The diluted virus is then further diluted with lyophilisation stabiliser (sucrose 4%, dextran 8%, sorbitol 6%, amino-acid 4%) up to the target viral titre, in this case $10^{5.6}$ ffu/dose. 0.5 ml aliquots of stabilised virus composition are aseptically transferred to 3 ml vials. Each vial is then partially closed with a rubber stopper, the sample is freeze dried under a vacuum, the vial is then fully closed and an aluminium cap is crimped in place around the vial to keep the stopper in place.

For use, the virus is reconstituted using one of the following antacid reconstituents:

(a) **Citrate reconstituent**

Sodium citrate is dissolved in water, sterilized by filtration and aseptically transferred into reconstituent containers in 1.5 ml amounts at a concentration of 544 mg Na$_3$Citrate.2H$_2$O per 1.5ml dose. The reconstituent containers may be for example 3 ml vials, or 4 ml vials, or 2 ml syringes, or soft plastic squeezable capsules for oral administration. As an alternative to maintaining sterile components under sterile conditions, the final container can be autoclaved.

(b) **Al(OH)$_3$** reconstituent

An aseptic aluminium hydroxide suspension (Mylanta - trademark) is aseptically diluted in sterile water, aseptically transferred to reconstituent containers (for example 2 ml syringes, or soft plastic squeezable capsules) in 2 ml amounts each containing 48 mg Al(OH)$_3$. An alternative to using sterile components under sterile conditions is to $\gamma$ irradiate the aluminium hydroxide suspension (preferably at a diluted stage).

Standard ingredients are included to prevent the suspension from settling. Such standard ingredients include for example magnesium stearate, carboxymethylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose, and silicone polymers.
Bacteriostatic agents for example butylparaben, propylparaben or other standard bacteriostatic agents used in food, and flavourings, may also be included.

2. Lyophilised virus with Al(OH)^+ in liquid formulation

Standard techniques are used for preparing virus doses. Frozen purified viral bulk is thawed and diluted with appropriate medium composition, in this case Dulbecco's modified eagle Medium, up to a desired standard viral concentration, in this case $10^{6.2}$ ffu/ml. Aluminium hydroxide suspension is added to reach a final quantity of 48 mg/dose and the virus composition is diluted with lyophilisation stabiliser (sucrose 4%, dextran 8%, sorbitol 6%, amino-acid 4%) up to the target viral titre, in this case $10^{5.6}$ ffu/dose. 0.5 ml aliquots of stabilised virus composition are aseptically transferred to 3 ml vials. Lyophilisation and closing of the vials is then carried out as described in part 1.

3. Lyophilised virus with Al(OH)$_2$ for blister presentation

Standard techniques are used for preparing virus doses. Frozen purified viral bulk is thawed and diluted with appropriate medium composition, in this case Dulbecco's modified eagle Medium, up to a desired standard viral concentration, in this case $10^{6.2}$ ffu/ml. Aluminium hydroxide suspension is added to reach a final quantity of 48 mg/dose and the virus composition is diluted with lyophilisation stabiliser which may be sucrose, dextran or amino-acid 4%, or gelatin, or vegetal peptone, or xanthane up to the target viral titre of $10^{5.6}$ffu/dose. An aseptic filling operation is employed to transfer doses of 0.5 ml or preferably less to blister cavities. The composition is lyophilised, and the blister cavities are sealed by thermic sealing.

Optionally standard ingredients are included to prevent the aluminium hydroxide suspension from settling. Such standard ingredients include for example magnesium stearate, carboxymethylcellulose, hydroxypropylmethylcellulose, microcrystalline cellulose, and silicone polymers. Flavourings may also be included.

Example 5: Rotavirus viral titration for various formulations

5.1 Comparison between lactose and sucrose based formulations:
Table 5

<table>
<thead>
<tr>
<th>Batch n°</th>
<th>Formulation composition</th>
<th>Viral titer before lyophilisation</th>
<th>Viral titer after lyophilisation and 1 week at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>98G06/01</td>
<td>Lactose:2%; Dextran:4%; Sorbitol:3%; AminoAcids:2%</td>
<td>10.522</td>
<td>10.467</td>
</tr>
<tr>
<td>98G06/03</td>
<td>Sucrose:2%; Dextran:4%; Sorbitol:3%; AminoAcids:2%</td>
<td>10.528</td>
<td>10.492</td>
</tr>
</tbody>
</table>

5 P43 rotavirus was formulated either with sucrose or with lactose as shown in the table above.

Viral titration before lyophilisation is the viral titre in the completed formulated liquid (containing sucrose dextran sorbitol aminoacids) and without the lyophilisation step.

Good results are those in which a <0.5 log decrease at the lyophilisation step and <0.5 log decrease during the “1 week at 37°C” (accelerated stability test) are achieved.

The precision of the viral titration is around + or - 0.2 log.

The results indicate that sucrose may be used instead of lactose.

5.2: Effect of arginine and replacement of sorbitol by maltitol:

Table 6

<table>
<thead>
<tr>
<th>Batch n°</th>
<th>Formulation composition</th>
<th>Viral titer at time = zero after lyophilisation</th>
<th>Viral titer after lyophilisation and 1 week at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>98L16/01</td>
<td>Lactose:2%; Dextran:4%; Sorbitol:3%; AminoAcids:2%</td>
<td>10.48</td>
<td>10.48</td>
</tr>
<tr>
<td>98L16/02</td>
<td>Lactose:2%; Dextran:4%; Sorbitol:3%; AminoAcids:2%</td>
<td>10.48</td>
<td>10.49</td>
</tr>
</tbody>
</table>
The results demonstrate that the addition of arginine (which is known to improve the stability of the virus during lyophilisation and also provides a basic medium in order to compensate for the stomach acidity) maintains the viral titer.

Sorbitol tends to decrease the glass transition temperature of the lyophilised cake by too great a degree. This can be overcome by using maltitol instead of sorbitol as shown above and the viral titer is still maintained.

5.3: Various formulation compositions
This experiment demonstrates that a number of formulations are possible.

Table 7
5.4: Association between Rotavirus and Al(OH)$_3$ antacid:

Table 8

<table>
<thead>
<tr>
<th>Rotavirus</th>
<th>Al(OH)$_3$</th>
<th>H$_2$O</th>
<th>Contact time at room temperature</th>
<th>Centrifugation</th>
<th>Supernatant viral titer in ffu/ml</th>
<th>Pellets viral titer in ffu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>10$^{5.6}$ ffu/ml</td>
<td>48 mg in 0.240ml</td>
<td>0.76 ml</td>
<td>30 min</td>
<td>8000rpm, 10 min</td>
<td>10$^{3.66}$</td>
<td></td>
</tr>
<tr>
<td>10$^{5.6}$ ffu/ml</td>
<td>0.48 mg in 0.240ml</td>
<td>0.76 ml</td>
<td>30 min</td>
<td>8000rpm, 10 min</td>
<td>10$^{4.41}$</td>
<td></td>
</tr>
<tr>
<td>10$^{5.6}$ ffu/ml</td>
<td>1 ml</td>
<td></td>
<td>30 min</td>
<td>8000rpm, 10 min</td>
<td>10$^{5.88}$</td>
<td></td>
</tr>
<tr>
<td>Rotavirus in Lyophilised Cake</td>
<td>12 mg in 0.120ml</td>
<td>1.38 0ml</td>
<td>30 min</td>
<td>8000rpm, 10 min</td>
<td>Below detection</td>
<td>10$^{4.7}$</td>
</tr>
</tbody>
</table>
Al(OH)$_3$ is used as an antacid. This shows that Rotavirus is associated with the insoluble inorganic salt (Al(OH)$_3$) since it centrifuged together with the Al(OH)$_3$ (decrease of viral activity in the supernatant).

5.5: Dissolution of Al(OH)$_3$ antacid by SodiumCitrate before viral titration

Table 9

<table>
<thead>
<tr>
<th>Viral samples</th>
<th>Dissolution</th>
<th>Conditions</th>
<th>Viral titers ffu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>99B10/06 liquid formulation before lyophilisation; $10^5$</td>
<td>1.5ml Na$_3$Citrate</td>
<td>24h at room temperature</td>
<td>$10^{5.11}$</td>
</tr>
<tr>
<td>99B10/06:lyophilised $10^5$</td>
<td>1.5ml Na$_3$Citrate</td>
<td>24h at room temperature</td>
<td>$10^{4.53}$</td>
</tr>
</tbody>
</table>

When Rotavirus is associated with the Al(OH)$_3$, it is possible to lyophilise everything (including the Al(OH)$_3$). After lyophilisation, it is possible to recover the Rotavirus by dissolving Al(OH)$_3$ in SodiumCitrate. This step does not damage the Rotavirus and retains its activity after this dissolution step.

5.6: Infectivity of Rotavirus after liberation of the Al(OH)$_3$-Rotavirus association:
The mechanism of virus liberation (by dissolution of the carrier) may very well occur in vivo. Indeed below pH 6, aluminium hydroxide becomes completely soluble, and thus, Rotavirus will be liberated in the stomach.

$$\text{Al(OH)}_3 + 3 \text{H}^+ \rightarrow \text{Al}^{+++} \text{ (water soluble)} + 3 \text{H}_2\text{O}$$


In the intestine, due to the increase of pH, insoluble forms of aluminium are precipitated (Al(OH)$_3$ or AlPO$_4$), and eliminated by the natural way.

It is unknown whether the newly formed Al(OH)$_3$ (or AlPO$_4$) precipitate will be able to re-associate with free Rotavirus. This raises the question of the infectivity of the Al(OH)$_3$-Rotavirus association itself.
Liberation of Rotavirus from the Al(OH)_3-Rotavirus association by other mechanisms is also possible. Lysine, for example, interferes with the viral adsorption on Al(OH)_3. Other anions like borate, sulfate, carbonate and phosphate are known to be specifically adsorbed on aluminium hydroxide, thus, theoretically, it should be possible to displace (by competition for the adsorption site) Rotavirus from the Al(OH)_3-Rotavirus association.

\[
\text{DRVC003A46} + 12\text{ mg Al(OH)3 in 0.120 ml} + 65\text{ mg Lysine 1.380 ml H2O} + 30\text{ min Room T.} + \text{Centrifugation 8000rpm 10 min} \\
\text{Culot} \quad \text{Supernatant} \\
\text{dissolution in Citrate}
\]

below detection 3.8

Thus, Rotavirus may be liberated from the Rotavirus - Al(OH)_3 association and the liberated Rotavirus remains active.

This liberation can be done either by dissolving Al(OH)_3 (by HCl in the stomach, or by Na_3Citrate in vitro) or by displacing Rotavirus by a basic amino acid (lysine).

5.7: Infectivity of the Al(OH)_3-Rotavirus association

A single dose of lyophilised Rotavirus was reconstituted with water and divided into two parts. The first part, considered as the reference, received an additional volume of water. The second part received 24mg of Al(OH)_3 suspended in 0.240 ml of water (Preclinical viral titrations).
When Al(OH)₃ is present, Rotavirus is active and the viral titration value is higher compared to the reference sample.

This experiment was repeated without dividing the lyophilised dose, and by adding 12 mg Al(OH)₃ or 24 mg Al(OH)₃.

Here the reference sample was the one reconstituted with a Citrate-Bicarbonate buffer. Thus, the viral titer is again higher in the presence of Al(OH)₃.
As in the example above, Rotavirus associates with the Al(OH)$_3$ particles, since the virus can be discarded by centrifugation. DRVC003A46 is a lyophilised formulated Rotavirus (Sucrose: 2%; Dextran: 4%, Sorbitol: 3%; Amino-acids: 2%).

\[
\begin{align*}
\text{DRVC003A46} & \quad \text{DRVC003A46} \\
+ & \quad + \\
12 \text{ mg Al(OH)}_3 & \quad 24 \text{ mg Al(OH)}_3 \\
in 0.120 \text{ ml} & \quad \text{in } 0.240 \text{ ml} \\
+ & \quad + \\
1.380 \text{ ml H}_2\text{O} & \quad 1.260 \text{ ml H}_2\text{O} \\
\text{Centrifugation} & \quad \text{Centrifugation} \\
8000 \text{ rpm 10 min} & \quad 8000 \text{ rpm 10 min} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Culot</th>
<th>Supernatant</th>
<th>Culot</th>
<th>Supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>SDSAA</td>
<td>SDSAA</td>
</tr>
<tr>
<td>5.78</td>
<td>&lt;1.44</td>
<td>5.92</td>
<td>&lt;1.44</td>
</tr>
<tr>
<td>5.96</td>
<td>&lt;1.44</td>
<td>6.11</td>
<td>&lt;1.44</td>
</tr>
</tbody>
</table>

SDSAA = Sucrose 2%, Dextran 4%, Sorbitol 3%, Amino-Acid 2%.

According to the viral titration carried out on the supernatant, the quantity of Al(OH)$_3$ needed to adsorb Rotavirus seems to be low (starting with one lyophilised dose 5.7 log) scaling up viral titration):

Table 10

<table>
<thead>
<tr>
<th>Al(OH)$_3$</th>
<th>Adsorption time</th>
<th>Titer in supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 mg</td>
<td>1 hour RT</td>
<td>2.7</td>
</tr>
<tr>
<td>24 mg</td>
<td>1 hour RT</td>
<td>3.4</td>
</tr>
<tr>
<td>48 mg</td>
<td>1 hour RT</td>
<td>3.4</td>
</tr>
<tr>
<td>72 mg</td>
<td>1 hour RT</td>
<td>2.0</td>
</tr>
<tr>
<td>96 mg</td>
<td>1 hour RT</td>
<td>Below detection</td>
</tr>
<tr>
<td>12 mg</td>
<td>Overnight</td>
<td>2.7</td>
</tr>
</tbody>
</table>
Time needed to adsorb Rotavirus on Al(OH)₃ seems to be short:
One dose of lyophilised Rotavirus was reconstituted in presence of 24 mg Al(OH)₃, and centrifuged after 0, 15, 60 min and 24 hours. The "culot" were resuspended in SDSAA before viral titration:

Table 11

<table>
<thead>
<tr>
<th>Time</th>
<th>Culot</th>
<th>Supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>5.26</td>
<td>3.17</td>
</tr>
<tr>
<td>15 min</td>
<td>5.34</td>
<td>&lt;1.44</td>
</tr>
<tr>
<td>60 min</td>
<td>5.96</td>
<td>&lt;1.44</td>
</tr>
<tr>
<td>24 hours</td>
<td>6.13</td>
<td>&lt;1.44</td>
</tr>
</tbody>
</table>

5.8: Using CaCO₃ as antacid
In order to avoid aluminium in the vaccine, the antacid Al(OH)₃ was replaced by another insoluble inorganic salt: CaCO₃ (calcium carbonate).

The phenomena observed with CaCO₃ are parallel to those described for Al(OH)₃:
- Association of Rotavirus with the inorganic salt;
- Maintainance of Rotavirus activity when associated with the inorganic salt;
- Possibility of liberation of Rotavirus from the association by dissolution of the inorganic base by an acid;
- Possibility of co-lyophilisation of the antacid and the Rotavirus.

CaCO₃ and Rotavirus association

In a first trial, lyophilised Rotavirus (viral titer 5.7) was reconstituted with a suspension of CaCO₃ in water (50mg in 1.5ml); and then centrifuged, and the viral titer of the supernatant compared to the pellet.
This indicates that more than 90% of the Rotavirus is associated with CaCO₃.

Also, when the virus was associated, it was possible to realise the titration and to recover the original viral quantities. Also, viral titers are slightly higher than those obtained without CaCO₃.

**Quantity of CaCO₃ and Rotavirus association**
Lyophilised Rotavirus was reconstituted with a CaCO$_3$ suspension in water (1.5ml):

- 10 mg
- 50 mg
- 100 mg

and then centrifuged, and the viral titer of the supernatant compared to the culot.

<table>
<thead>
<tr>
<th>CaCO3</th>
<th>Extempo + Centri.</th>
<th>1 Hour + Centri</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culots</td>
<td>Surpernatant</td>
</tr>
<tr>
<td>100mg</td>
<td>4.57</td>
<td>3.01</td>
</tr>
<tr>
<td>50mg</td>
<td>4.17</td>
<td>4.15</td>
</tr>
<tr>
<td>10mg</td>
<td>3.17</td>
<td>4.77</td>
</tr>
</tbody>
</table>

Thus, clearly, more CaCO$_3$ and more virus is associated, and less is found in the supernatant. However, the full dose is not completely recovered (expected a total of 5.3 at least or even 5.8 as obtained earlier - see above).

**CaCO$_3$ Protection of Rotavirus during Baby Rossett-Rice antacid titration**

Using 10 doses of lyophilised Rotavirus (DRVC003A46) and 50mg of CaCO$_3$, two types of baby Rossett-Rice titration were carried out:

In a classic Rossett-Rice titration, the antacid is mixed with Rotavirus and HCl is poured into this medium.

In the "inverse" baby Rossett-Rice, the situation is the reverse: antacid is dropped into the HCl pool (as it occurs in vivo).

<table>
<thead>
<tr>
<th>Classical baby Rossett-Rice titration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophi. Rota stored at.</td>
</tr>
<tr>
<td>4°C</td>
</tr>
<tr>
<td>-80°C</td>
</tr>
<tr>
<td>4°C</td>
</tr>
</tbody>
</table>
Thus, in this *in vitro* experiment, calcium carbonate is able to protect about 20% of Rotavirus from the presence of HCl, while aluminium hydroxide is not able to.

### 5.9: Lyophilisation of Rotavirus in presence of CaCO₃ antacid:

Table 14

<table>
<thead>
<tr>
<th>Batch n°</th>
<th>Composition</th>
<th>Viral titer at time = zero after lyophilisation</th>
<th>Viral titer after lyophilisation and 1 week at 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>99K08/01</td>
<td>Sucrose: 2% Dextran: 4% Sorbitol: 3% Am. Acids: 2% CaCO₃: 50 mg</td>
<td>$10^{5.28}$</td>
<td>$10^{5.10}$</td>
</tr>
<tr>
<td>99K08/02</td>
<td>Sucrose: 2% Dextran: 4% Sorbitol: 3% Am. Acids: 2% CaCO₃: 60 mg</td>
<td>$10^{5.16}$</td>
<td>$10^{5.15}$</td>
</tr>
<tr>
<td>00C24/01</td>
<td>Sucrose: 2% Dextran: 4% Sorbitol: 3% Am. Acids: 2% CaCO₃: 60 mg Xanthane 0.3%</td>
<td>$10^{5.07}$</td>
<td>$10^{4.69}$</td>
</tr>
<tr>
<td>00C24/03</td>
<td>Sucrose: 2% Dextran: 4% Sorbitol: 3% Am. Acids: 2% CaCO₃: 60 mg Xanthane 0.3%</td>
<td>$10^{5.07}$</td>
<td>$10^{4.85}$</td>
</tr>
<tr>
<td>00E09/25</td>
<td>Sucrose: 2% Dextran: 4%</td>
<td>$10^{5.03}$</td>
<td>$10^{4.91}$</td>
</tr>
</tbody>
</table>
This is the “all in one” lyophilisation of Rotavirus and antacid (CaCO₃) together in the same vial. To prevent sedimentation of CaCO₃ during the filling step, viscous agents are needed. Examples of such viscous agents include Xanthane gum and Starch. The Rotavirus activity is maintained even in the presence of Xanthane gum and Starch.

5.10 Lyophilised tablets for quick disintegration when placed in the mouth:
The following formulations demonstrate the "lyoc" concept. That is, quick dissolution of the lyophilised cake in the mouth.

<table>
<thead>
<tr>
<th>Batch n°</th>
<th>Formulation composition</th>
<th>Viral titer before lyophilisation</th>
<th>Viral titer after lyophilisation and 1 week at 37°</th>
</tr>
</thead>
<tbody>
<tr>
<td>99B10/06</td>
<td>Sucrose 4% Sodium glutamate 3.7% Al(OH)₃ 48mg</td>
<td>10⁵¹¹</td>
<td>10⁴⁵³</td>
</tr>
<tr>
<td>99C11/12</td>
<td>Maltitol 3% Al(OH)₄ 48mg Hydroxypropylmethylcellulose: 1%</td>
<td>10⁴¹⁶</td>
<td>10³⁷⁹</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch n°</th>
<th>Formulaion composition</th>
<th>Viral titer at time = zero after lyophilisation</th>
<th>Viral titer after lyophilisation and 1 week at 37°</th>
</tr>
</thead>
</table>
In the "lyoc concept" both Xanthane and Starch can be used (maintaining the quick dissolution properties of the lyophilised cake).

| 00C24/05 | Sucrose: 2%  
| Dextran: 4%  
| Sorbitol: 3%  
| Am. Acids: 2%  
| CaCO₃: 60 mg  
| Xanthane 0.3% | 10⁵.02 | 10⁴.54 |
| 00C24/06 | Sucrose: 2%  
| Dextran: 4%  
| Sorbitol: 3%  
| Am. Acids: 2%  
| CaCO₃: 60 mg  
| Xanthane 0.3% | 10⁴.86 | 10⁴.56 |
| 00F26/11 | Sucrose: 1%  
| Dextran: 2%  
| Sorbitol: 1.5%  
| Am. Acids: 1%  
| CaCO₃: 60 mg  
| Starch: 2% | 10⁴.70 | 10⁴.40 |

EXAMPLE 6: Use of Calcium Carbonate as the antacid for the Rotavirus vaccine composition

When a suspension of CaCO₃ in water is used as the antacid for Rotavirus there is a problem that the calcium carbonate particles sediment rapidly when placed in water since the powder density value approaches 2.6 and the average particle size is 30µm. This sedimentation can be slowed by:

1. increasing the density of the surrounding medium
2. increasing the viscosity of the surrounding medium
3. reducing the particles size
4. keeping particles away from each other

6.1: Increasing density of the surrounding medium:
When the CaCO₃-Water suspension (when placed in the syringe) is placed on the lyophilised cake (containing sucrose 2%, dextran 4%; sorbitol 3%; amino-acids 2%) the
density of the surrounding medium is increased, but the speed of CaCO$_3$ sedimentation is not very much different from the CaCO$_3$-Water suspension.

6.2 Increasing the viscosity of the surrounding medium:

Pseudoplastic excipients
A pseudoplastic solution is defined as a solution having higher viscosity on standing compared to its viscosity under agitation.

Usual excipients of this type are:

- **natural polymers for example:**
  - arabic gum
  - adragante gum
  - agar-agar
  - alginites

- **semi-synthetic polymers for example:**
  - carboxymethylcellulose (Tyloses C®)
  - methylcellulose (Methocels A®, Viscotrans MC®, Tylose MH® and MB®)
  - hydroxypropylcellulose (Klucels®)
  - hydroxypropylmethylcellulose (Methocels E® and K®, Viscotrans MPH®)

In general those pseudoplastic excipients are used together with thixotropic agents.

Pseudoplastic excipients with low flowing capacity
Those polymers, at a sufficient concentration, give rise to a structural fluid arrangement resulting in a high viscosity solution having low flowing capacity on standing. A certain quantity of energy needs to be given to the system to allow flowing and transfer. External energies (agitation) are needed to destroy temporarily the structural fluid arrangement in order to obtain a fluid solution.

Examples of such polymers are Carbopols® and Xanthane gum.

Thixotropic excipients
With these excipients, on standing, a gel structure is obtained; while under agitation a fluid solution is obtained.

Examples of thixotropic excipients are: Veegum © (Magnesium-aluminium silicate) and Avicel RC® (about 89% microcrystalline cellulose and 11% Carboxymethylcellulose Na).

6.3 Reducing the particles size
A reduction in the CaCO_3 particle size resulted in a decrease in the antacid capacity of the compound.

6.4 Keeping particles away from each other
This is the case in Veegum® and Avicel® for which insoluble particles smaller (about 1 \( \mu \)m) than the CaCO_3 particles, are placed between CaCO_3 particles in order to prevent aggregation.

EXAMPLE 7: Product design
The following schemes demonstrate examples of possible product designs.

7. 1 CaCO_3 in the syringe

Having already clinical batches of Rotavirus in lyophilised vials, the antacid can be placed in the reconstituent liquid contained in the syringe.

![Syringe with 1.3 ml CaCO_3 (60mg/ml) needle lyophilised Rotavirus](image)
In this product presentation, sedimentation of CaCO$_3$ must be under control not only during the filling steps, but also during the complete shelf-live of the product (at least 2 years).

7.2 CaCO$_3$ in the lyophilised vial

Lyophilised vial Rotavirus + CaCO$_3$ (60mg) Xanthane

EXAMPLE 8: Lyophilisation of different strain of Rotavirus

<table>
<thead>
<tr>
<th>Batch n°</th>
<th>Rotavirus strain</th>
<th>Formulation composition</th>
<th>Viral titer at t = zero after lyophilisation</th>
<th>Viral titer after lyophilisation and 1 week at 37$^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>00F26/01</td>
<td>G1 SB purif n°61</td>
<td>Sucrose: 2% Dextran: 4%</td>
<td>$10^{4.8}$</td>
<td>$10^{4.7}$</td>
</tr>
</tbody>
</table>
The strains DS-1, P and VA70 are described as Human rotavirus reference strains for serotype G2, G3 and G4 respectively at page 1361 of "Fields" Raven press 1990, second edition.

In this experiment different Rotavirus strains have been lyophilised. For all, both the viral titer have been maintained during lyophilisation and accelerated stability (one week at 37°C) has been shown.

**EXAMPLE 9: Phase I safety study in adults of one oral administration of the Rotavirus vaccine.**

A Phase I study was carried out to assess the safety and reactogenicity of a single oral dose of $10^{6.0}$ ffu of the P43 vaccine in healthy adults aged 18 to 45 years.

The clinical trial was double blind and randomized. It was placebo-controlled and self-contained. The study was performed in one single centre in Belgium.

**9.1. Study Population**

A total of 33 subjects, 11 in the placebo group and 22 in the vaccine group, were enrolled and all completed the study. All volunteers were Caucasians. Their mean age at the time of vaccination was 35.3 years, with a range of 18 to 44 years. The trial began in January and ran for just over one month.
9.2. Material

Vaccine
Clinical lots of P43 vaccine were produced, purified, formulated and lyophilized according to Good Manufacturing Practices. The lots were released by Quality Control and Quality Assurance. Each vial of vaccine contained the following components:

Active ingredient:
P43 strain Min. $10^{5.8}$ ffu

Excipients, stabilizers:
Sucrose 9 mg
Dextran 18 mg
Sorbitol 13.5 mg
Amino acids 9 mg

Placebo
Vials of placebo were prepared and released. Each vial of placebo contained the following components:

Excipients, stabilizers:
Sucrose 9 mg
Dextran 18 mg
Sorbitol 13.5 mg
Amino acids 9 mg

Diluent
Water for injection was used as diluent to reconstitute vaccine and placebo.

9.3. Administration

Approximately 10 to 15 minutes before administration of the vaccine or the placebo, subjects of both groups were given 10 ml of Mylanta® orally. Mylanta® is a registered antacid. The antacid increases the pH of the stomach and prevents inactivation of the rotavirus during its passage through the stomach.

To prepare the vaccine, two vials of lyophilized P43 containing $10^{5.8}$ ffu per vial were reconstituted with 1.5 ml of diluent water for injection. This achieved a calculated viral
titer of $10^6$ ffu per dose. The reconstituted vaccine was administered promptly as a single oral dose.

To prepare the placebo, two vials of lyophilized placebo were reconstituted with 1.5 ml water for injection and administered orally as a single dose.

9.4. Safety and Reactogenicity

The following criteria of safety and reactogenicity applied:

Solicited general symptoms were fever, diarrhea, vomiting, nausea, abdominal pain and loss of appetite. They were recorded during eight days post administration.

Unsolicited symptoms were recorded during 30 days post administration.

Serious adverse events were recorded during the entire study period.

Diarrhea samples were to be collected during eight days post administration.

The results were:

No solicited symptoms, no unsolicited and no serious adverse events were reported during the respective observation periods.

No cases of diarrhea were reported.

9.5. Conclusions

SB Biologics P43 vaccine was safe relative to the placebo when administered orally in a double-blind fashion as a single dose at the dose of $10^6$ ffu to healthy adult volunteers aged 18 to 44.

EXAMPLE 10 - Efficacy of two doses of a human monovalent Rotavirus vaccine, containing RIX 4414 in preventing Gastro-enteritis due to G1 and non-G1 (G9) Rotavirus

10.1. Methods

A randomised, double-blind, placebo-controlled phase II trial was conducted in Latin America to evaluate the protective efficacy of a vaccine (RIX4414 human rotavirus strain) derived from the G1P[8] human strain 89-12 for infant immunisation. RIX4414 vaccine comprises as rotavirus component the attenuated G1P[8] human strain deposited as ECACC deposit 99081301 (WO 01/12797).
**Vaccine composition** (Table 17)
The HRV vaccine or placebo was prepared for administration by injecting the entire content of one pre-filled syringe containing the calcium carbonate buffer into the vial of the lyophilized product (vaccine or placebo). The vial was shaken to resuspend the vaccine/placebo. The entire volume of the resuspended product was withdrawn into the same syringe, the needle discarded and the resuspended product administered promptly as a single oral dose (approximately 1.0 ml).

**Table 17 - RIX4414 rotavirus vaccine composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (per nominal dose: 1 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active ingredient</strong></td>
<td></td>
</tr>
<tr>
<td>RIX4414</td>
<td>$10^{58}$ ffu/dose</td>
</tr>
<tr>
<td><strong>Excipients</strong></td>
<td></td>
</tr>
<tr>
<td>Lyophilized vaccine in glass vial</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>9 mg</td>
</tr>
<tr>
<td>Dextran</td>
<td>18 mg</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>13.5 mg</td>
</tr>
<tr>
<td>Amino acids</td>
<td>9 mg</td>
</tr>
<tr>
<td>Dulbecco’s Modified Eagle Medium (DMEM)</td>
<td>2.25 mg</td>
</tr>
<tr>
<td><strong>Liquid diluent (CaCO$_3$-based) in pre-filled syringe</strong></td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>60 mg</td>
</tr>
<tr>
<td>Xanthan</td>
<td>2.5 mg</td>
</tr>
<tr>
<td>Water for Injections</td>
<td>q.s. ad 1 ml</td>
</tr>
</tbody>
</table>

**Vaccine administration**
Healthy infants (493) received two doses of the RIX4414-rotavirus vaccine at a viral concentration of $10^{58}$ ffu per dose, or placebo (504) at age 2 and 4 months, concomitantly with DTPw-HBV and Hib vaccines. Three doses of OPV (oral polio virus vaccine) were given 2 weeks apart from study vaccine, i.e. were not to be administered during the period starting 2 weeks before each dose of study vaccine and ending 2 weeks after. Two other groups received 2 doses of the RIX4414-rotavirus vaccine at different viral concentrations: $10^{47}$ ffu and $10^{52}$ ffu. Diarrhoeal samples were tested for the presence of rotavirus (ELISA) and the serotypes determined in positive samples (RT-PCR). Diarrhoeal episodes reported from two weeks after the second dose were considered for the efficacy analysis. Severity was determined using a 20-point scale.
(Ruuska and Vesikari, 1990). The 20-point scoring system used to assess the severity of each diarrhoea episode in this study is shown below in Table 18. A score ≥ 11 defined severe disease.

### Table 18

<table>
<thead>
<tr>
<th>Adverse Experience</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration of looser than normal stools (days)</strong></td>
<td></td>
</tr>
<tr>
<td>1–4</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>≥ 6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Maximum number of looser than normal stools/24 hours</strong></td>
<td></td>
</tr>
<tr>
<td>1–3</td>
<td>1</td>
</tr>
<tr>
<td>4–5</td>
<td>2</td>
</tr>
<tr>
<td>≥ 6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Duration of vomiting (days)</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>≥ 3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Maximum number of episodes of vomiting/24 hours</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2–4</td>
<td>2</td>
</tr>
<tr>
<td>≥ 5</td>
<td>3</td>
</tr>
<tr>
<td><strong>Fever (measured rectally/axillary)</strong></td>
<td></td>
</tr>
<tr>
<td>37.1–38.4°C/36.6–37.9°C</td>
<td>1</td>
</tr>
<tr>
<td>38.5–38.9°C/38.0–38.4°C</td>
<td>2</td>
</tr>
<tr>
<td>≥ 39°C/≥ 38.5°C</td>
<td>3</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
</tr>
<tr>
<td>Rehydration</td>
<td>1</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>2</td>
</tr>
<tr>
<td><strong>Dehydration</strong></td>
<td></td>
</tr>
<tr>
<td>1–5 %</td>
<td>2</td>
</tr>
<tr>
<td>≥ 6%</td>
<td>3</td>
</tr>
</tbody>
</table>

* The highest temperature recorded during the episode was scored.

#### 10.2. Results

An interim analysis of efficacy was performed on the above mentioned group and the isolated serotypes were mainly G1 and G9, almost evenly distributed. The overall attack rate in the placebo group varied from 4.8% for G1 to 3.6% for G9 during the 6 months observation period. Two doses of RIX4414 rotavirus vaccine at $10^{5.8}$ ffu protected against all types of diarrhoea caused by G1 with 83% efficacy [95% CI: 50.4-95.7] and 92.1% efficacy [95% CI: 47.6-99.8] against severe gastro-enteritis. If the diarrhoea was
caused by G9, the protection against all types of diarrhoea was 60.2 % [95% CI: 0.2-86.0] and 80.8% [95% CI: 33.0-96.4] against severe gastro-enteritis. For each of these efficacy endpoints (any and severe for G1 and G9), there was a statistically significant decrease in diarrhoea episodes in the HRV group as compared to the placebo group (p < 0.05, two-sided Fisher’s exact test).

The results obtained in the other 2 vaccine groups (different rotavirus concentration) are consistent with those reported in the Example, and are presented in the final analysis (Example 11). Efficacy data for G2, G3 and G4 were also analysed. No conclusion from this study was drawn about G2, G3 and G4 cross-protection as too few cases were reported. However data of efficacy against G2, G3 and G4 are presented in the final analysis on a more important sample size (Example 11).

10.3. Conclusion.
These results are highly supportive of the efficacy of 2 doses of a monovalent HRV vaccine, RIX4414 rotavirus vaccine in protecting young infants against G1 strain and cross-protect against the G9 strain.

EXAMPLE 11 - Efficacy of two doses of a human monovalent Rotavirus vaccine, containing RIX4414 strain, administered at three different virus concentrations in preventing Gastro-enteritis due to G1 and non-G1 (G2, G3, G4, G9) Rotavirus

11.1. Methods
A randomised, double-blind, placebo-controlled phase II trial was conducted in Latin America to evaluate the protective efficacy and efficacy against hospitalization of a vaccine derived from the G1P[8] human strain 89-12 for infant immunisation. Specifically the vaccine used was named RIX4414 rotavirus vaccine, and comprises as the rotavirus component the attenuated G1 human strain deposited as ECACC deposit 99081301.

Healthy infants received two doses of RIX4414 rotavirus vaccine at three different virus concentrations. The cohort for efficacy analysis consisted of 1846 subjects (468 subjects in the 10^{47} ffu HRV vaccine group, 460 subjects in the 10^{52} ffu HRV vaccine group, 464 subjects in the 10^{58} ffu HRV vaccine group and 454 subjects in the placebo group at age 2 and 4 months, concomitantly with DTPw-HBV and Hib vaccines. Three doses of OPV were given 2 weeks apart from study vaccine, i.e. were not to be administered during the
period starting 2 weeks before each dose of study vaccine and ending 2 weeks after. Diarrhoeal samples were tested for the presence of rotavirus (ELISA) and the serotypes determined in positive samples (RT-PCR). Diarrhoeal episodes reported from two weeks after the second dose until subjects were one year of age were considered for the efficacy analysis. Severity was determined using a 20-point scale (Ruuska and Vesikari, 1990). A score ≥ 11 defined severe disease (see Example 10 for the description of the 20-point scoring system).

11.2. Results
Results which are the final analysis of the data mentioned in Example 10 are illustrated in the tables below. Infants in the vaccine groups had significantly fewer rotavirus gastroenteritis episodes than children in the placebo group (p < 0.001, two-sided Fisher's exact test) (Table 19). Depending on the dosage, protective efficacy against severe rotavirus gastroenteritis reached 85.6% (95%CI: 63.0%-95.6%), and 70% (95%CI, 45.7%-84.4%) against any rotavirus gastroenteritis (Table 20). For each of these efficacy endpoints, there was a statistically significant decrease in diarrhoea episodes in the HRV group as compared to the placebo group (p < 0.001, two-sided Fisher's exact test).

Multiple rotavirus serotypes (G1, G2, G3, G4 and G9) were identified from gastroenteritis stools (ELISA and RT-PCR) allowing to also calculate vaccine efficacy against non-G1 serotypes. As can be seen from Table 21 in particular, for non-G1 serotypes (G2, G3, G4 and G9), and depending on the dosage, efficacy against severe rotavirus gastroenteritis reached 82.7% (95%CI: 40.3%-96.8%), providing proof of concept that the monovalent G1-based G1P1A P[8] human rotavirus vaccine elicits cross-protection against heterotypic (i.e. non-G1 and non-P[8]) strains.

Table 19: Features of rotavirus gastro-enteritis episodes reported during the study

<table>
<thead>
<tr>
<th></th>
<th>RIX4414 10^47 ffu</th>
<th>RIX4414 10^52 ffu</th>
<th>RIX4414 10^58 ffu</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any rotavirus gastroenteritis</td>
<td>21</td>
<td>22</td>
<td>15</td>
<td>51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>no. of episodes (percent) with specific feature among all rotavirus gastroenteritis episodes reported</th>
<th>RIX4414 10^47</th>
<th>RIX4414 10^52</th>
<th>RIX4414 10^58</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>4 (19.0)</td>
<td>8 (36.4)</td>
<td>2 (13.3)</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>7-10</td>
<td>5 (23.8)</td>
<td>4 (18.2)</td>
<td>8 (53.3)</td>
<td>12 (23.5)</td>
</tr>
<tr>
<td>≥ 11</td>
<td>12 (57.1)</td>
<td>10 (45.5)</td>
<td>5 (33.3)</td>
<td>34 (66.7)</td>
</tr>
</tbody>
</table>
### Identified rotavirus serotypes

<table>
<thead>
<tr>
<th></th>
<th>wild GI</th>
<th>12 (57.1)</th>
<th>6 (27.3)</th>
<th>7 (46.7)</th>
<th>30 (58.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>0</td>
<td>0</td>
<td>1 (6.7)</td>
<td>3 (5.9)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>1 (4.8)</td>
<td>0</td>
<td>0</td>
<td>2 (3.9)</td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>0</td>
<td>0</td>
<td>1 (6.7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>G9</td>
<td>8 (38.1)</td>
<td>14 (63.6)</td>
<td>7 (46.7)</td>
<td>15 (29.4)</td>
<td></td>
</tr>
<tr>
<td>Canine</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(2.0)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>2 (9.1)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

### Table 20: Protective efficacy of two doses of RIX4414 human rotavirus vaccine against rotavirus gastroenteritis

<table>
<thead>
<tr>
<th></th>
<th>Any rotavirus gastroenteritis</th>
<th>Severe rotavirus gastroenteritis</th>
<th>Hospitalization for rotavirus gastroenteritis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n (%)</td>
<td>Efficacy (95% CI)</td>
</tr>
<tr>
<td>Pooled vaccine groups</td>
<td>1392</td>
<td>58 (4.2)*</td>
<td>61.4 (42.3;4.1)</td>
</tr>
<tr>
<td>RIX4414 10^58 ffu</td>
<td>464</td>
<td>15 (3.2)*</td>
<td>70.0 (45.7;84.4)</td>
</tr>
<tr>
<td>RIX4414 10^52 ffu</td>
<td>460</td>
<td>22 (4.8)*</td>
<td>55.7 (25.3;74.5)</td>
</tr>
<tr>
<td>RIX4414 10^47 ffu</td>
<td>468</td>
<td>21 (4.5)*</td>
<td>58.4 (29.4;76.3)</td>
</tr>
<tr>
<td>Placebo</td>
<td>454</td>
<td>49 (10.8)</td>
<td>-</td>
</tr>
</tbody>
</table>

*p<0.001 for each comparison between the vaccine and placebo groups by two-sided Fisher's exact test (significant level of α=0.05)

| t =0.037 for the comparison between the vaccine and placebo groups by two-sided Fisher's exact test (significant level of α=0.05)

| 10 | N = number of subjects
n/% = number/percentage of subjects reporting at least one specified rotavirus gastroenteritis episode

Exact 95% confidence intervals are shown
Table 21: Protective efficacy of two doses of RIX4414 human rotavirus vaccine against serotype specific severe rotavirus gastroenteritis

<table>
<thead>
<tr>
<th>Severe rotavirus gastroenteritis</th>
<th>N</th>
<th>n(%)</th>
<th>Efficacy (95% CI)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G1 wild type rotavirus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled vaccine groups</td>
<td>1392</td>
<td>13 (0.9)</td>
<td>73.5 (41.2;88.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RIX4414 10^58 ffu</td>
<td>464</td>
<td>2 (0.4)</td>
<td>87.8 (48.0;98.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RIX4414 10^52 ffu</td>
<td>460</td>
<td>4 (0.9)</td>
<td>75.3 (23.5;94.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>RIX4414 10^47 ffu</td>
<td>468</td>
<td>7 (1.5)</td>
<td>57.6 (-9.0;85.2)</td>
<td>0.057</td>
</tr>
<tr>
<td>Placebo</td>
<td>454</td>
<td>16 (3.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Non-G1 rotavirus (mainly G9 with G2, G3 and G4 types)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled vaccine groups</td>
<td>1392</td>
<td>14 (1.0)</td>
<td>73.1 (42.1;87.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RIX4414 10^58 ffu</td>
<td>464</td>
<td>3 (0.6)</td>
<td>82.7 (40.3;96.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>RIX4414 10^52 ffu</td>
<td>460</td>
<td>6 (1.3)</td>
<td>65.2 (7.4;88.8)</td>
<td>0.020</td>
</tr>
<tr>
<td>RIX4414 10^47 ffu</td>
<td>468</td>
<td>5 (1.1)</td>
<td>71.5 (19.4;91.8)</td>
<td>0.009</td>
</tr>
<tr>
<td>Placebo</td>
<td>454</td>
<td>17 (3.7)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Two-sided Fisher’s exact test (significant level of α=0.05) used for each comparison between the vaccine and placebo groups.

N = number of subjects
n/ (%) = number/percentage of subjects reporting at least one specified severe rotavirus gastroenteritis episode
Exact 95% confidence intervals are shown

11.3. Conclusion
These results are highly supportive of the efficacy of 2 doses of a monovalent HRV vaccine containing RIX4414, in protecting young infants against any and severe rotavirus gastroenteritis caused by the G1 strain and broad cross-protection against other RV G types, namely G2, G3, G4 and G9.

EXAMPLE 12 - Two doses of the Human Attenuated Rotavirus vaccine RIX4414 vaccine show heterotypic protection in Latin America and Europe
The Efficacy of a 2 dose oral, live attenuated G1P[8] human rotavirus (RV) vaccine containing RIX 4414 strain was analysed in a Phase II/III clinical trials in Finnish and
Latin American infants. RIX 4414 rotavirus vaccine comprises as the rotavirus component the attenuated G1 human strain deposited as ECACC deposit 99081301.

12.1. Methods
Part of the results of Example 12 is already presented in Examples 10 and 11. Data were pooled from Phase II studies, one in Finland and one in Latin America (Brazil, Mexico and Venezuela) (Examples 10 and 11) and from one Phase III study in 11 Latin American countries (Example 13) using the same methodology and efficacy criteria. In total, 20081 healthy infants (cohort for efficacy) vaccinated with 2 doses of RIX 4414 vaccine or placebo at 2 and 4 months of age were followed until one year of age for severe gastroenteritis (GE) with a score on the Vesikari (Ruuska T et al. Scand. J. Infect. Dis. 1990, 22, 259-267) severity scale ≥ 11. GE samples were tested for rotavirus (by ELISA) and typed by RT-PCR.

A meta analysis was conducted on the three mentioned studies. Pooled efficacy for severe RV GE (defined as Vesikari severity score ≥ 11) was calculated from 2 weeks post-dose 2 to 1 year of age (adjustment for study effect using the Mantel-Haenszel approximation).

12.2. Results
In the cohort for efficacy 5 severe rotavirus GE episodes of G2P[4] type with a Vesikari score ≥ 11 were detected in the vaccine group and 13 episodes in the placebo group. Vaccine efficacy against the G2P[4] type was 67.2% (95% CI: 14.8; 87.1), which shows that in addition to protecting against homotypic strains (G1P[8], G3P[8] and G4P[8]), RIX4414 vaccine protects against severe rotavirus GE caused by the heterotypic non-P[8] non-G1 G2P[4] strain.

Type-specific efficacy across the different studies is given below (Table 22).

Table 22

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of severe RV cases</th>
<th>% Vaccine Efficacy* (VE) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Vaccines (N = 10646)</td>
<td>N Placebo (N = 9435)</td>
</tr>
</tbody>
</table>
Only 3 G4 cases occurred in all 3 trials, 1 in vaccine and 2 in placebo recipients.

12.3. Conclusion
This analysis shows that, in addition to giving a high level of protection against homologous G1 rotavirus strains (which have two outer capsid proteins (VP4 and VP7) and one inner capsid protein (VP6) antigenically similar to the vaccine), RIX 4414 vaccine is also highly protective against other strains which have either a different G type (eg G3, G9), a different P type (eg P[4]), or both different G type and P type, as illustrated by the efficacy against G2P[4].

**Example 13 - Meta analysis showing that two doses of the Human Attenuated Rotavirus vaccine RIX 4414 show heterotypic protection**

As more data became available from Singapore and from a European study (Example 15), an additional meta analysis was carried out to include these studies in addition to studies mentioned in Example 12.

13.1. Methods
Three phase II (Finland and Latin America and Singapore) and two phase III studies (Latin America and Europe) were included in the meta-analysis. Two oral doses were administered according to 0,1 to 2 month schedule to healthy infants who were 6-14 weeks of age at Dose 1. In all studies, severe RVGE was defined as a score ≥11 on the 20-point Vesikari scale. Diarrhoeal samples were analyzed for the presence of RV by ELISA and typed by RT-PCR based method. Efficacy against any RV GE was evaluated in the three phase II studies and the phase III Europe study only as in the phase III Latin America study, only severe RV GE were recorded.

<table>
<thead>
<tr>
<th>G Type</th>
<th>N</th>
<th>RRVGE</th>
<th>VEับ</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>17</td>
<td>52</td>
<td>83.7</td>
<td>(70.0; 91.2)</td>
</tr>
<tr>
<td>G2P[4]</td>
<td>5</td>
<td>13</td>
<td>67.2</td>
<td>(14.8; 87.1)</td>
</tr>
<tr>
<td>G3</td>
<td>2</td>
<td>8</td>
<td>82.7</td>
<td>(5.9; 96.8)</td>
</tr>
<tr>
<td>G9</td>
<td>15</td>
<td>34</td>
<td>79.5</td>
<td>(59.2; 89.7)</td>
</tr>
</tbody>
</table>

*VE adjusted for study effect using Mantel-Haenszel approximation
VE and its 95%CI was estimated as 1-rate of RVGE relative to placebo using exact Poisson rate ratio stratified by study (Proc StatXact4 for SAS Users, 1999, cytel software corporation, exact Confidence Interval for common relative risk, p298)

13.2. Results
In a total of 8221 infants vaccinated with two doses of RIX4414 or placebo, 4 episodes of any G2P[4] RVGE were detected in the RIX4414 (N=5783) and 9 episodes in the placebo (N=2438) group, indicating a VE of 81.0% (95% CI:31.6;95.8) against RVGE of any severity due to G2P[4] strain.

In a total of 26088 healthy infants vaccinated with two doses of RIX4414 or placebo, 6 episodes of severe RVGE due to G2P[4] type were detected in the RIX4414 (N=14792) and 15 episodes in the placebo (N=11296) group, indicating a VE of 71.4% (95% CI:20.1;91.1) against severe RVGE due to G2P[4] strain. Results are reported in Table 23.

Table 23 - Number of subjects reporting any or severe RVGE episodes caused by G2P[4] RV type and percentage of vaccine efficacy during the first efficacy period - (meta analysis), cohort for efficacy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Any RV GE* (Latin America excluded)</th>
<th>Severe RV GE (score ≥11 on Vesikari scale) (5 studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>% VE</td>
<td>95% CI</td>
</tr>
<tr>
<td>G2P[4]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRV vaccine</td>
<td>5783</td>
<td>4</td>
</tr>
<tr>
<td>Placebo</td>
<td>2438</td>
<td>9</td>
</tr>
</tbody>
</table>

N = number of subjects included in each group;
n/% = number/percentage of subjects reporting at least one specified RV G2P[4] GE episode in each group;
% VE = observed vaccine efficacy,
95%CI = 95%Confidence Intervals
* Two out of 13 G2 were not P typed
13.3. Conclusion:
This meta analysis on vaccine efficacy against G2P[4] RV type shows a vaccine efficacy of 81.0% (95% CI: 31.6%; 95.8%) against any RV GE due to G2P[4] type and a vaccine efficacy of 71.4% (95% CI: 20.1%; 91.1%) against severe RV GE due to G2P[4] type.

EXAMPLE 14 - Efficacy of Human Attenuated Rotavirus vaccine Rotarix™ in a multi-country phase III trial

14.1. Methods
20169 healthy infants from 11 Latin American countries were to receive two oral doses of HRV vaccine (10159) or placebo (10010) at approximately 2 and 4 months of age. Stool specimens were tested for rotavirus (RV) by ELISA and typed by RT-PCR using suitable primers and type-specific probes. The clinical case definition for capture of severe gastroenteritis episode was an episode of diarrhea (passage of three or more looser than normal or watery stools within 24 hours) with or without vomiting that required overnight hospitalization and/or rehydration therapy equivalent to WHO plan B (oral rehydration therapy) or WHO plan C (intravenous rehydration therapy) in a medical facility such as hospital, clinic or supervised rural health care center (http://www.who.int/criilcl-adolescent-health/New_Publications/CHILDHEALTH/textrev4.htm). Disease severity was graded using the 20-point Vesikari scale; severe RVGE was defined as a score \( \geq 11 \). Vesikari's score was modified: Since the dehydration was not recorded in the eCRF, the following rule was applied: a subject that had a severe GE episode was considered as being dehydrated between 1 to 5% if this subject received oral re-hydration. A subject was considered as being dehydrated \( \geq 6 \% \) if the subject was hospitalized and/or received intravenous (IV) re-hydration.

14.2. Vaccine efficacy

*Vaccine efficacy against severe rotavirus gastroenteritis* (Table 24)
The cohort for efficacy consisted of 9009 subjects vaccinated with HRV vaccine and 8858 subjects receiving a placebo recipient. There were 12 children with severe rotavirus gastroenteritis according to the clinical definition in the vaccine and 77 in the placebo group (2.0 vs. 13.3 children with \( \geq 1 \) episode per 1,000 child-years, respectively; \( p<0.001 \), two-sided Fisher's exact test), resulting in a vaccine efficacy of 84.7% against
severe rotavirus gastroenteritis from 15 days post-dose 2 until one year of age (shown in Table 24). Similar results were obtained with the total vaccinated cohort (vaccine efficacy of 81.1%; 95 % C.I. 68.5-89.3; p < 0.001, two-sided Fisher's exact test) from dose 1 until one year of age. Hospitalization for at least one night was required in 9 children in the vaccine and 59 in the placebo group (1.5 vs. 10.2 hospitalizations per 1,000 child-years, respectively), for a vaccine efficacy against hospitalization for severe rotavirus gastroenteritis of 85 % (p<0.001, two-sided Fisher's exact test) (Table 24).

Table 24 - Vaccine efficacy against rotavirus severe gastroenteritis, specific rotavirus G types severe gastroenteritis and all-cause severe gastroenteritis, during the period from two weeks after dose 2 until one year of age

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>Placebo group</th>
<th>RR</th>
<th>Vaccine efficacy (95% CI) and p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N = 9,009)</td>
<td>(N = 8,858)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>1,000 infants-year ratio</td>
<td>n</td>
<td>1,000 infants-year ratio</td>
</tr>
</tbody>
</table>

Severe rotavirus gastroenteritis according to the clinical case definition

<table>
<thead>
<tr>
<th>Rotavirus gastroenteritis</th>
<th>Vaccine group</th>
<th>Placebo group</th>
<th>RR</th>
<th>Vaccine efficacy (95% CI) and p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe gastroenteritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>12</td>
<td>2.0</td>
<td>77</td>
<td>13.3</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>9</td>
<td>1.5</td>
<td>59</td>
<td>10.2</td>
</tr>
</tbody>
</table>

All-cause gastroenteritis                                    |               |               |    |                                      |
| Severe gastroenteritis                                        | 183           | 30.9          | 300| 51.7 | 0.600 | 40.0 (27.7; 50.4) | <0.001 |
| Hospitalization                                              | 145           | 24.5          | 246| 42.4 | 0.580 | 42.0 (28.6; 53.1) | <0.001 |

Type specific gastroenteritis                                |               |               |    |                                      |
| G1P[8]#                                                     | 3a            | 0.5           | 36b| 6.2  | 0.082 | 91.8 (74.1; 98.4) | <0.001 |
| G3P[8], G4P[8], G9P[8]                                     | 4c            | 0.66          | 31d| 5.3  | 0.126 | 87.3 (64.1; 96.7) | <0.001 |
| G2P[4]                                                     | 6             | 1.0           | 10e| 1.7  | 0.590 | 4.10 (-79.2; 82.4) | 0.328 |

Severe rotavirus gastroenteritis with a score ≥ 11 on the Vesikari scale
### Table 24: Vaccine efficacy according to Vesikari score

<table>
<thead>
<tr>
<th>Type specific gastroenteritis</th>
<th>Vaccine group (N = 9,009)</th>
<th>Placebo group (N = 8,858)</th>
<th>RR</th>
<th>Vaccine efficacy (95% CI) and p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1P[8]¹</td>
<td>n</td>
<td>1,000 infants-year ratio</td>
<td>n</td>
<td>1,000 infants-year ratio</td>
</tr>
<tr>
<td>G1P[8]</td>
<td>3</td>
<td>0.5</td>
<td>32</td>
<td>5.5</td>
</tr>
<tr>
<td>G3P[8], G4P[8], G9P[8]</td>
<td>4</td>
<td>0.66</td>
<td>30</td>
<td>5.2</td>
</tr>
<tr>
<td>G2P[4]</td>
<td>5</td>
<td>0.8</td>
<td>9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Legend to Table 24:

Participants with episodes with more than one isolated G type were counted in each of the detected rotavirus type category.

n = number of infants reporting at least one specified episode

RR = Relative Risk = ratio of the incidence rate of subjects reporting at least one episode in the vaccine group over the incidence rate of subjects reporting at least one episode in the placebo group.

CI = confidence interval

The 1000-infant year ratio is the number of infants presenting with >= 1 specified episode per infant-year

*Case definition according to the study protocol: an episode of diarrhea (passage of three or more looser than normal or watery stools within a day) with or without vomiting that required overnight hospitalization and/or rehydration therapy equivalent to WHO plan B (oral rehydration therapy) or WHO plan C (intravenous rehydration therapy) in a medical facility such as hospital, clinic or supervised rural health care center.

¹ All G1 types isolated were wild-type rotavirus; G1P[8] and G9P[8] were isolated from one infant

² G1P[8] type alone was isolated from 2 infants; G1P[8] and G9P[8] were isolated from one infant

³ G1P[8] type alone was isolated from 34 infants; G1P[8] and G9P[8] were isolated from one infant; G1, G2, G9 types were isolated from one infant

⁴ G3P[8] type alone was isolated from one infant; G4P[8] type alone from 1 infant; and G9P[8] alone from one infant; G1P[8] and G9P[8] were isolated from one infant

⁵ G3P[8] type alone was isolated from 8 infants, G4P[8] type alone from 2 infants; and G9P[8] alone from 19 infants; G1P[8] and G9P[8] were isolated from 1 infant; and G1P[8] and G2P[4 and G9P[8] from 1 infant

⁶ G2P[4] alone was isolated from 9 infants and G1P[8], G2P[4] and G9P[8] were isolated from 1 infant

p_values = two-sided Fisher’s exact test (significant level of α=0.05)

Vaccine efficacy according to Vesikari score
Eleven of 12 children with severe rotavirus episodes in the vaccine group and 71 of 77 in the placebo group had Vesikari score $\geq 11$, resulting in a vaccine efficacy of 84.7% (P<0.001, two-sided Fisher's exact test). For increasing disease severity with scores between 11 and 20, vaccine efficacy was increasingly higher, reaching 100% against more severe rotavirus gastroenteritis. A total of 16 severe rotavirus gastroenteritis episodes with Vesikari score $\geq 11$ were reported from dose 1 until dose 2, six in the vaccine group and 10 in the placebo group.

**Vaccine efficacy according to Vesikari score per rotavirus type**

Type specific vaccine efficacy against wild-type strains is shown in Table 24. Vaccine efficacy against severe rotavirus episodes with Vesikari score $\geq 11$ caused by G1P[8] type strains, homologous to the vaccine strain, was 91.8% (P<0.001, two-sided Fisher's exact test). Vaccine efficacy against strains sharing the P[8] antigen (G3P[8], G4P[8] and G9P[8]) was 86.9% (P<0.001, two-sided Fisher's exact test). G2P[4] rotavirus type, which is not sharing either the G nor the P antigen with the vaccine strain was detected in five episodes in the vaccine and nine in the placebo group, resulting in an efficacy of 45 percent (P=0.298, two-sided Fisher's exact test). Because of the small number of G2 episodes observed in this study, a meta-analysis of 5 studies (Example 13) was performed and the trend observed in this study has become a significant value when the results of the 5 studies were pooled. (Example 13)

**Vaccine efficacy on the burden of diarrhea illness**

Children with gastroenteritis of any cause requiring hospitalization and/or rehydration according to WHO plan B/C had an incidence rate of 30.9/1,000 child-years in the vaccine compared to 51.7 in the placebo group, for an overall 40 % (P<0.001, two-sided Fisher's exact test) reduction in severe diarrhea episodes of all cause among vaccine recipients. Likewise, hospitalization for diarrhea of any etiology was significantly reduced by 42 % (P=0.001, two-sided Fisher's exact test) (Table 24, all causes GE)).

**14.3. Summary of results**

Vaccine efficacy against severe rotavirus gastroenteritis (RV GE) and against rotavirus associated-hospitalization was 85% (P<0.001, two-sided Fisher's exact test), reaching 100% in a population having RV GE with a Vesikari's score $\geq 19$. Efficacy against G1P[8] and strains sharing only the P[8] epitope with HRV was 92% (95% C.I. 74.98) and
Example 15 - Efficacy of Human Attenuated Rotavirus vaccine Rotarix™ in six European countries

15.1. Methods

3,994 children in six European countries were randomized to receive 10^{6.5} CCID_{50} HRV (human rotavirus) vaccine Rotarix™ (see composition) or placebo when co-administered with routine childhood vaccinations. The first efficacy follow-up period started from two weeks after Dose 2 and ended June - July 2005. A total of 3874 subjects were part of the 1\textsuperscript{st} year efficacy cohort.

Vaccine composition: (Table 25)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSK Biologicals' HRV vaccine</td>
<td>RIX4414 HRV strain derived from the 89-12 HRV vaccine strain 106.5 median Cell Culture Infective Dose (CCID50) Dulbecco's Modified Eagle Medium (DMEM) 3.7 mg Sucrose 9 mg Dextran 18 mg Sorbitol 13.5 mg Amino acids 9 mg</td>
</tr>
<tr>
<td>GSK Biologicals' diluent</td>
<td>Calcium carbonate 80 mg Xanthane 3.25 mg Water for injection q.s. ad 1.3 ml</td>
</tr>
</tbody>
</table>

15.2. Vaccine efficacy

The HRV vaccine was highly effective in protecting against RV GE during the first efficacy period. Vaccine efficacy was 87.1\% (95\% CI: 79.6\%; 92.1\%) against any episodes of RV GE and 95.8\% (95\% CI: 89.6\%; 98.7\%) against severe RV GE episodes. For increasing disease severity (Vesikari scores between 11 and 20), vaccine efficacy was increasingly higher, reaching 100\% in a population having RV GE with a Vesikari
score ≥ 17 points. Vaccine efficacy against hospitalization for RV GE was 100% (95% Cl: 81.8%; 100%) and against RV GE episodes requiring medical attention was 91.8% (95% Cl: 84.0%; 96.3%) (Tables 26 and 27).

Table 26 - Percentage of subjects reporting any and severe RV GE, percentage of subjects hospitalized due to RV GE episodes, and vaccine efficacy during first efficacy period - cohort for efficacy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Any RV GE</th>
<th>Severe RV GE (score ≥ 11 on Vesikari scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subjects</td>
<td>Vaccine efficacy</td>
</tr>
<tr>
<td>1 × 10⁶ CCID 50</td>
<td>2572</td>
<td>24</td>
</tr>
<tr>
<td>Placebo</td>
<td>1302</td>
<td>94</td>
</tr>
</tbody>
</table>

Hospitalized RV GE

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Vaccine efficacy</th>
<th>%</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 × 10⁶ CCID 50</td>
<td>2572</td>
<td>0</td>
<td>0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Placebo</td>
<td>1302</td>
<td>12</td>
<td>0.9</td>
<td>-</td>
</tr>
</tbody>
</table>

N = number of subjects included in each group
N% = number/percentage of subjects reporting at least one specified RV GE episode in each group
P-value = two-sided Fisher’s exact test (significant level of α=0.05)
% VE = observed vaccine efficacy
95%CI = 95% Confidence Intervals
a score ≥ 11 on the 20-point Vesikari scale was defined as severe

Table 27 - Percentage of subjects reporting RV GE episodes requiring medical attention and vaccine efficacy during the first efficacy period - cohort for efficacy

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>RV GE requiring medical attention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>1 × 10⁶ CCID 50</td>
<td>2572</td>
<td>10</td>
</tr>
<tr>
<td>Placebo</td>
<td>1302</td>
<td>62</td>
</tr>
</tbody>
</table>

N = number of subjects included in each group;
The HRV vaccine was highly protective against any and severe RV GE caused by G1P[8], G3P[8], G4P[8] and G9P[8] strains (Table 28). Protection against G2P[4] RV type that does not share any of the outer capsid antigens of the HRV vaccine was lower in this study however the results of a meta analysis taking into account phase II and III efficacy studies showed a significant protective efficacy against any and severe GE due to G2P[4] (see Example 13).

Table 28 - Percentage of subjects reporting any or severe RV GE episodes and vaccine efficacy by serotype during the first efficacy period - cohort for efficacy

<table>
<thead>
<tr>
<th>Groups</th>
<th>RV Strain</th>
<th>Viral Conc</th>
<th>N</th>
<th>n</th>
<th>%</th>
<th>Any RV GE</th>
<th>P-value</th>
<th>Severe RV GE (score ≥11 on Vesikari scale)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vaccine efficacy</td>
<td>95%CI</td>
<td>Vaccine efficacy</td>
<td>95%CI</td>
</tr>
<tr>
<td>G1 P[8] Wild type</td>
<td>10⁶.⁵ CCID₅₀</td>
<td>Placebo</td>
<td>2572</td>
<td>4</td>
<td>0.2</td>
<td>95.6</td>
<td>87.9-98.8</td>
<td>&lt;0.001</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1302</td>
<td>46†</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2 P[4]</td>
<td>10⁶.⁵ CCID₅₀</td>
<td>Placebo</td>
<td>2572</td>
<td>4</td>
<td>0.1</td>
<td>62.0</td>
<td>-124.4-94.4</td>
<td>0.234</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1302</td>
<td>4</td>
<td>0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3 P[8]</td>
<td>10⁶.⁵ CCID₅₀</td>
<td>Placebo</td>
<td>2572</td>
<td>5</td>
<td>0</td>
<td>89.9</td>
<td>9.5-99.8</td>
<td>0.018</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1302</td>
<td>5</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4 P[8]</td>
<td>10⁶.⁵ CCID₅₀</td>
<td>Placebo</td>
<td>2572</td>
<td>3</td>
<td>0</td>
<td>88.3</td>
<td>57.5-97.9</td>
<td>&lt;0.001</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1302</td>
<td>13†</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G9 P[8]</td>
<td>10⁶.⁵ CCID₅₀</td>
<td>Placebo</td>
<td>2572</td>
<td>13</td>
<td>0.5</td>
<td>75.6</td>
<td>51.1-88.5</td>
<td>&lt;0.001</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1302</td>
<td>27</td>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pooled non G1 P[8] (G2, G3, G4, G9)</td>
<td>10⁶.⁵ CCID₅₀</td>
<td>Placebo</td>
<td>2572</td>
<td>20</td>
<td>0.8</td>
<td>79.3</td>
<td>64.6-88.4</td>
<td>&lt;0.001</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>1302</td>
<td>49</td>
<td>3.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = number of subjects included in each group;
n/% = number/percentage of subjects reporting at least one RV GE episode in each group;
P-value = two-sided Fisher's exact test (significant level of α=0.05);
% VE = observed vaccine efficacy,  
95%CI = 95% Confidence Intervals 
a score ≥11 on the 20-point Vesikari scale was defined as severe 
t One subject from the Placebo group counted in G1 and G4 categories since both 
serotypes were isolated

15.3. Summary of results
Two oral doses of HRV Rotarix™ vaccine, co-administered with childhood vaccinations, 
were highly effective during the first efficacy period compared to the placebo in 
protecting infants against any RV GE caused by G1P[8] wild-type RV and by non-
G1P[8] RV types, vaccine efficacy was 95.6% (95% CI: 87.9%; 98.8%) and 79.3% (95% 
Cl: 64.6%; 88.4%) respectively. The efficacy against severe RV GE caused by G1P[8] 
wild-type RVs and by non- G1P[8] RV types is 96.4% (95% CI: 85.7%; 99.6%) and 
95.4% (95% CI: 85.3%; 99.1%) respectively.

These results are very supportive towards the conclusion that the HRV vaccine provides 
broad coverage against circulating RV strains (see Table 28: G1P[8], G2 P[4], G3P[8], 
G4P[8], G9P[8]). A meta analysis on vaccine efficacy against G2 P[4] specifically was 
performed, please refer to Example 13.

Overall Conclusions

The RIX4414 rotavirus vaccine proved to be highly protective against rotavirus 
gastroenteritis episodes measured by a clinical definition for case capture focusing on 
hospitalization and re-hydration, as well as by the validated Vesikari scale which 
includes quantifiable morbidity outcomes related with diarrhea, vomiting, fever, 
dehydration and hospitalization. Two oral doses of HRV vaccine were highly efficacious 
in protecting infants against any and severe RVGE and hospitalization due to multiple 
circulating rotavirus strains.

A high level of protection was demonstrated against homologous G1P[8] rotaviruses, 
which have two outer capsid proteins (VP4 and VP7) and one inner capsid protein (VP6) 
antigenically similar to the HRV vaccine. It also protected well against strains sharing 
only the genotype P[8] (VP4 antigen) and the VP6 antigen. Protection against rotavirus 
strains not sharing any of the outer capsid antigens of the HRV vaccine was also 
demonstrated in a meta analysis including the results of three phase II studies from 
Finland, Singapore and Latin America (all using identical methodology and efficacy
criteria) and of 2 phase III studies from Latin America and Europe, and which are reported in Example 13, vaccine efficacy against G2P[4] type of any severity was 81% (95% C.I. 31.6-95.8) and vaccine efficacy against severe GE due to G2P[4] type was 71.4% (95 percent C.I. 20.1-91.1) indicating that the vaccine can also protect against strains which do not share identical G or P proteins with the vaccine strain.
Claims

1. A method of inducing an immune response against rotavirus strain, the method comprising administering to a subject a composition comprising an attenuated rotavirus strain of a GxPy type, said composition generating an immune response against a rotavirus strain which is neither a Gx nor a Py type.

2. Use of an attenuated rotavirus strain from GxPy type in the manufacture of a medicament for inducing an immune response against rotavirus infection caused by a rotavirus strain which is neither a Gx nor a Py type.

3. A method or use according to any preceding claim wherein the composition comprises a rotavirus having a VP4 gene comprising, in the nucleotide sequence, at least one of the following: an adenine base (A) at position 788, an adenine base (A) at position 802 and a thymine base (T) at position 501 from the start codon.

4. A method or use according to claim 3 in which the VP4 gene comprises a nucleotide sequence comprising an adenine base (A) at positions 788 and 802 and a thymine base (T) at position 501 from the start codon.

5. A method or use according to any of claims 1-4 in which the composition comprises a rotavirus having a VP7 gene comprising, in the nucleotide sequence, at least one of the following: a thymine (T) at position 605, an adenine (A) at position 897 and a guanine (G) at position 897 from the start codon.

6. A method or use according to claim 5 in which the VP7 gene comprises a nucleotide sequence comprising a thymine (T) at position 605 and an adenine (A) or a guanine (G) at position 897 from the start codon.

7. A method or use according to any of claims 1 to 6, wherein the composition comprises a rotavirus having a VP4 gene comprising, in the nucleotide sequence, an adenine (A) at positions 788 and 802 and a thymine (T) at position 501 from the start codon; and wherein the VP7 gene comprises, in the nucleotide sequence, a thymine (T) at position 605 and an adenine (A) at position 897 from the start codon.
8. A method or use according to any of claims 1 to 7 wherein the composition comprises a G1 rotavirus strain and is used to induce an immune response to G1 and at least one of the non-G1 serotypes selected from the group consisting of: G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13 and G14 serotypes.

9. A method or use according to claim 8 wherein the composition comprises a G1 rotavirus strain and is used to induce an immune response to the G1 and G2 serotypes.

10. A method or use according to claim 9 wherein the composition is additionally able to induce an immune response to at least one of the non-G1 serotypes selected from the group consisting of: G3, G4 and G9.

11. A method or use according to any of claims 1 to 10 wherein the composition comprises a P[8] rotavirus strain and is used to induce an immune response to P[8] and at least one of the non-P[8] types selected from the group consisting of: P[1], P[2], P[3], P[4], P[5], P[6], P[7], P[9], P[11], P[12], P[14] and P[19] types.

12. A method or use according to claim 11 wherein the composition is used to induce an immune response to the P[4] type.


14. A method or use according to any preceding claim wherein the composition comprising an attenuated rotavirus strain of a GxPy type provides protection against severe rotavirus-induced gastroenteritis caused by infection of a rotavirus strain which is neither a Gx nor a Py type.

15. A method or use according to any of claims 1 to 14 wherein the composition comprising an attenuated rotavirus strain of a GxPy type is at least 40% protective, in a population of vaccinated individuals, against diarrhoea caused by a rotavirus strain.
which is neither a Gx nor a Py type of a rotavirus strain which is neither a Gx nor a Py
type.

16. A method or use according to claim 15 wherein the composition is at least 50%
protective.

17. A method or use according to any preceding claim wherein the composition is at
least 60% protective.

18. A method or use according to any of claims 14 to 17 wherein the composition is
between 40% and 80% protective.

19. A method or use according to claim 18 wherein the composition is between 50%
and 70% protective.

20. A method or use according to any of claims 1 to 19 wherein the composition
comprises a G1P[8] rotavirus strain which is between 40% and 75% protective in a
population of vaccinated individuals against severe gastro-enteritis caused by infection
of rotaviruses with a G2P4 serotype.

21. A method or use according to any of claims 1 to 20 wherein the rotavirus strain in
said composition is ECACC deposit 99081301, or is obtainable or derivable from
ECACC deposit 99081301.

22. A method or use according to any of claims 1 to 21 wherein the composition is
administered in a 2-dose regime.

23. A method or use according to any of claims 1 to 21 wherein the attenuated
rotavirus strain is formulated with a suitable pharmaceutical carrier or with an antacid
buffer or both.
FIG 1A VP4 sequence of P43

ATGCGCTTCAC TCATTTATAG ACAACTTCTC ACTAATTCAT ATTCAGTAGA 50
TTATCATGAT GAAATAGGAC AAATGGGATC AGAAAAACT CAGAAGTAA 100
ATAAATATCC GGTCCTGATT GACACAGCTA GATGATGGCC AGTCTAAGTG 150
GATCAGGAG AGATATAAGT TCTGCTACA GTGAAACCAA TTTTGAGTTG 200
TCCTTATGCT CCACTACAT TTACCTCAC ATCTGATTAT TGAGTACTTT 250
TTATATTCATA TACAAATGGA GTAGTTATAT AAGATCACCA TAATATGAC 300
TTTGGACTTG CAGTGTGTTG TATTGACCC CACGTCACCC CAGTAGATAG 350
ACATATATAG ATATGGGCTG AAAGCAAGCA ATTTAATGTT ATGGAACTAGT 400
CAATATAATTG GAAGTTTCTTA GAAAATTTTA GAGGCACATT TCAAAAATGAA 450
TTTATATATA GAGTCAGTGA AACATCTGAT ACCAGACTTG TAGGAAATAT 500
TAATATATGGT GGAAGAGATT GGACATTTCA TTGGGAAACA CGGAGACGTA 550
CTACGACAG CTTCAAGTCT GCAAATTTAA TAAATATATC AATTACATTT 600
CATTCCAGAT TTACATTATG TCCAAGTTTT CAGAATCTTA AATGTAATGA 650
ATATTTATAT AATGGTGCTC CACCAATTCA AATACATGAA AATGGTGTTT 700
CATATTTGAT CTATCTGAG TCCATACAGT ATATAGAGAC ACAAGTTAAAT 750
GAAGACATTT TAGTCTCAAA AACTCTAATA TGGAAAGAAA TGGATGATAA 800
TAGGATTATA ATTAATGATG TTAATATTTG TAAATATATG GTAAGATGTT 850
GAGGACTTTT GTATTATTTTG TCTGAAATAT CATATAAGGC AGCAATTATT 900
CAATATATATG CACTACTGTG CGGTGCAACA GTAAACCCTG ACCACACTTG 950
TTTAGTGAAAT GGAATGCAAA ATTTATGCTA TAAATGAGGAG TTTTCTACCCCA 1000
CTGATTTGGG TATATTTCAA TGAGAGTTA TTAAGAGAAA TTCCTATATGTA 1050
TATTTATGAC ATTTGAGATG TTCCAAAGCA TTAAGAAATA TGGATGATGTT 1100
TAGATGTATTG CAGCTTATTG AAATATGTTA GAAATGCTAC AGTGGAAGTT 1150
ATTATTTTTG TAGCTCCATG GCTCCTACTTGG CGTGAAAGAA TGGTGCGCTT 1200
GTTTGGTTTG ATTTTGCGG AGTTGACATT TCCACGGAAT TTACTGATTT 1250
TGGATTTTAT AATCTCAATT GATTTGAGA GTGATATGAC TGGATGAC 1300
CACCTTTCTC AAATCTGAGA ACACGTCAGA TGAATTTGTA TGGATGATCA 1350
CCGCGGTATA CAAATATTGT AAATTTAATC TACAGAAAAT CAGAAGTATT 1400
CTGTATCTTT TCTTATGTTA AACAATGAGA TAGGATACAG ACGCTTAAT 1450
TGATTTATAG GAGCTGAAAG CAAAGTTTAG AGCGCGACAT TATCTATTTA 1500
CGAGAAGGTT TTTACTATTG GCACAAAGAA ATATAGCTTG CACAGTTATT 1550
TAATATATTG CTGTTGCTTTC TAGATATTTA TTTGCTATTTG TCAGAGATTA 1600
AAATGTACAG TGAAGTTGCT AAATCAAGGC GCAGCTAGTG AATAGGAAAGA 1650
TTTGAGAAAA CAAATGTTAG TACACATATT TCAGAAGATG TAAATATGTTA 1700
GTCAAGTCTC GCTTCATGAG CATCGAAAGA GCTTTCTATT AGATCAGAAT 1750
TATGCGCTGT TCCAAATGTTG ACTATGTTTT CAAATAGATG GTCAAGCTGA 1800
ACTAATATAT GAAAGCTATA CTAGCTTCAAA ATATTCTACAA TTTGAGGAA 1850
ACTTAGGTA AAAGAAATGTT TTACCTCAAC TGAGGAGGAT GCTTGTTGAAG 1900
ACATTTTACG AGCTTGACTA AAAAACAAA AAATAGTATG TACTCAAAAT 1950
GCGGAAATTA CTTATCTCTG TATATTCTAC GAAGCATTGA AAGAAATTAT 2000
TCCAAAGGCA TCTATGGAAG TATAGAAAGA TGATGGATGA TAAATATGAA 2050
ATACTGGAGA AAATTTTTCT GCACAAAGAA TTAATACATT TGATGGATGT 2100
CCACGATGTC ATAAATGAAA GCCTGACTGA GTTACAGATT CTCAGTTATT 2150
ATCACGGATA ATGATTTTTA AGACCTGAGA AAATTTAATT GAAATTTATTG 2200
GAATCAGTCT TACAAGAGT TAAATTTTAA TTAAATGCAA TCCAAATATG 2250
TTTAGTATTT TCTATATGGC AAATATATCA ATTAATGAGA ATGAGTTGGA 2300
ACAGTATAATA CTGCAATCTA AATTGTTGGA AGCTCATTTG GAAGTGAC 2350
FIG 1B VP4 sequence of P43 (RIX4414 VP4; 2359 bp)

```
1  ggctataaa TGCTTCGCT ACTTATATAG CAACTTCTCA CTAATTCATA TCCAGTAGAT
61  TTACATATGTG AAAATAGAGA AATTGGTACA GAAAACACT AGATAATGAA TATATATCCCG
121  GATCCATTTG CAGAGCTAGT ATATGTCCTCA GATCAATTGGG ACTAGGAGAG GATAAATTATG
181  TCGACTACAG GAGAAGAGGT CCTATATATGG CACTTACATT TACTCCACCT
241  AATGGATTAT GGTATTATAT TATCATATAT ACAATATGAG TAGTATATGAG AGTAAATACAT
301  AATAGTGTACT TTGGAGTCTG AGGTGTGTTGG ATGAAACCGC AGCTACACCC AGTAGATAGA
361  CAAATATATGG AGCAAGAGAA TTTAAATGTA GCTGAACTAT CAAATAAAATG
421  AAGTGTATTAG GAAATTTGTA AAGCATATAT GAAAGTATGG TATATAATAG ACCTTACTAT
481  AACTCTGTATA CCAGACTTGT AGGATATATC AAAATATGGT GAAGAGATAG GACATTCCAT
541  GGTGAAACAC CGAGAGCTAC TACTGCACTT TCAATGACTG CAAATTTAAA TAATATATAC
601  ATTTACAGTT ATCCAGATTT TATCATTATT CAAAGTTCCC AGGAAATCAA ATGTAATGAA
661  TATATATATA ATGATGTTCC CAAACATCAA ATATAGAAAT ATGAGATTTCC ATGATCAATTA
721  TCCATCTAGAT CGATATAGTT TAAGAGGACA CAAGTAATGC AAGCATAATT AGTATTCAAAA
781  AACTTATTAT GGAAGAAGAT CGATATATAG AATGGAAGGT TCTATTCCAT TATAGTTTGG
841  AATGATAGTT TAAGAAATATG TATCATATGG ATGAGAATCT CTAATGAGTA TGGGTGGATG
901  CAAATATTTTC ATATAGTTATG CTTATATATG AGTATATAGT CGATATATAG CAAATTTTGA
961  TCGATATGTT GAGAAATGTA TTTTATGTAC ATGATATAGA AATGGAAGGT TCTATTCCAT
1021  AATGATAGTT TAAGAAATATG TATCATATGG ATGAGAATCT CTAATGAGTA TGGGTGGATG
1081  CAAATATTTTC ATATAGTTATG CTTATATATG AGTATATAGT CGATATATAG CAAATTTTGA
1141  AATGATAGTT TAAGAAATATG TATCATATGG ATGAGAATCT CTAATGAGTA TGGGTGGATG
1201  GGTGAAATGG GCTGAATGTA TTTATGTTATG ATGATATAGA AATGGAAGGT TCTATTCCAT
1261  GTATCATTAA ATTCCTACTG ATATAGATTT GATAGGACAG TTGATGAAAC AACTTTTCTCA
1321  ATACTGAGAA CACTCTGATG GAATTTAGTA GATAAGGTTA CCGTACGTT AATAAATGGA
1381  AGTAACTTAC AGAAGAGATG TCTCTAGCTA CTATGATTTT TCTTTAGGCT AACTTATTAC
1441  GTATTACAGA TCTCTAGGTA AGTAACTTAC AGAAGAGATG TCTCTAGCTA CTATGATTTT
1501  AGTAACTTAC AGAAGAGATG TCTCTAGGTA AGTAACTTAC AGAAGAGATG TCTCTAGCTA
1561  GATTATACGAC TCTCTAGGTA AGTAACTTAC AGAAGAGATG TCTCTAGCTA CTATGATTTT
1621  GATTATACGAC TCTCTAGGTA AGTAACTTAC AGAAGAGATG TCTCTAGCTA CTATGATTTT
1681  ACCTACATTG CAGACAGGAA ATATCATTCC TCAATGAGTT CTCATCTACG ATCAAGAAGAC
1741  GTTCTATCTA GAACAGATGG CTTTATCTGA CTTTATCTGA CTTTATCTGA CTTTATCTGA
1801  TCAAACTAGAA CTATCTGATG GAAAGATGG CAGCAGAGGAA AATCAAGAATG TATGGAAGGA
1861  CTTATGCTTAA AAGAAATGTT TACCTCAACT GAAAGATGG CAGCAGAGGAA AATCAAGAATG
1921  GCTGACTGAA AAGAAATGTT TACCTCAACT GAAAGATGG CAGCAGAGGAA AATCAAGAATG
1981  AATGATAGTT TAAGAAATATG TATCATATGG ATGAGAATCT CTAATGAGTA TGGGTGGATG
2041  AGTAACTTAC AGAAGAGATG TCTCTAGGTA AGTAACTTAC AGAAGAGATG TCTCTAGCTA
2101  GATGAAAGTT CATTCTGATT AATATTTAAC CGGTGGGATT CTTCTATGTA CTTCTATGTA
2161  TCAAACTAGAA CTATCTGATG GAAAGATGG CAGCAGAGGAA AATCAAGAATG TATGGAAGGA
2221  AGAAGAGATG TCTCTAGGTA AGTAACTTAC AGAAGAGATG TCTCTAGCTA CTATGATTTT
2281  AATATTTAAC CGGTGGGATT CTTCTATGTA CTTCTATGTA CTTCTATGTA CTTCTATGTA
2341  cgctattgag gatgtgacc
```
Fig 2A  VP7 sequence of P43

ATGTATGGTC TTGAATATAC CACAATTCGA ATCTTTTCTGA TATCAATTAT 50
TCTACTCAAC TATATATTAA AATCAGTAC TCGAATAATG GACTACATA 100
TATGATGGAT CTTTGGAGAT TATGTGACAT TATTTGCCCT GACAAGAGCT 150
CAGATTATTG GGTGTTAAGT TTTTACTACCA CTTAATAACA GCATTGTATA 200
CGCTAACTCT ACTCAGAGAG CATAATTTTCT CAACTGCCCA TTATGTCTTG 250
ATTATCAAAC TGAAGCAAGT ACTCAAAATT AATGAGTGGTA ATGGAAGAC 300
TCATGTGCTC AACATTTTGA ATATAGAAGT TGGCCACAGG CAGATGCTCA 350
TTTTAAAGAG TTTTCAAGTA TTTGTGATTT TTCTGTGGAT CTACAAATTAT 400
ATTGAGTTTA TAACTTGGTA CTAATGAAAT ATGTAATAAA ACTTCCATTA 450
GATTATGTCG TATTTGCGTA TTATATTATG AATGAAATGG TATGTAATCC 500
AATGGATATA ACATATTATT ATATTCAACA ATCGGAGAAA TCAATTAAAG 550
GGATATCAAT GGATACATCA TGTAATGCTG AAGTTGTCGC ACTGAATACG 600
CAAATGTATA GTAATGTTTG TCAATAAACA ATATGAGACT GTTTGGAAT 650
GGTGCTGAG AATTGGAATT AGCTATGATT GCATTGCTTG GATGGAATAA 700
ATCAAAAATT AAATTGGACA ACTACGACAT GTACTATTCC AAATTTGAG 750
AAATGATGTC CAAGAGGAAA TGTATTGCTA ATACAGTGAT GTCGCGATTA 800
TGATTAGAC ATACAGCAG ATCCAAAGAC TAATCCACAA ATGAGGAAGA 850
TGATGAGATT GGGATAAAGA AATTTGGGCG AATATTTTTA TACTATAGTA 900
GATTTATATG ACCAAATGCG CAGAGTATAT CCAAAGAGAT CRAATAGTAA 950
AAATTCGCTA GCTTTTATT AATAGAGTATA GATATATCCT AGATTAGATC 1000
GATGGAAC

Fig 2B  VP7 sequence of P43 - (RIX44414 VP7; 1046 bp)

1 gcgtcttgaa ccgattaatg tattatagtc aaccggtata gcctttttaa ATGATGGTATT
61 GAAATTAACCA CAATCTAATTC TTCTTTGATA TAATATTCCC ATCTACTCA AATAATTTAAA
121 TCAGTAACCT GCAGATTGCA AGAACTATT AATATACATT GTGTGAGTTA TGATGCAATT
181 TTGTGCTCGA CAAAGCTCTC GAATTTGCCC CTTAACATCA CAATCAGGAT GCATTGACAG
241 ACTGTATAGG CCAAATGACCA ATTCAGAAGA ATATTTTTAA CACATCCATG ATGTTTTTGTAT
301 TATCCAAATGT AAACGGAGTG TGGGACTGAA ATATACAGT GATGGTGAGA GGAAGACCTG AATGGAACAT
361 ATGTTTTTCCAA AAAAAAGGTT CGGCAAGACG TCGATCTATG TTAAGAGGTA CAAAAGTATT
421 GTGAGTTTTTT CTTGTGAGTTT ACAATATTATT ATGTGATATT ACTATGACTA AATGGAATAT
481 GATCAAAATTTT TTAATTTTGA TTTTTCGGTTA TTTATTTGAA TAAATTGGA TAAATTGGA
541 TGGAAATCCAA TTGATATAAT GATATATATG TATCAACAAAT CCGGAGAAATC AAATGAGTTG
601 ATATCAATGTG GATCATCAGT TACTTGAAAGA GTTGACAGCA TGACAAGGCA AATGGAATAG
661 ATAGAGTTGTC AAACAAAGAA TTTGACTGGT AATTTAATAA TATTTTAAATG CAAAGAATT
721 GCATATTGGC ATGTTGATGGT TGGAGATAAT TATATAATTTG ATAGTACAGA TACAGTACTG
781 ACTATCTGAA CACAGAGAAT CATTGGTCACT AAGAGAAATG TAGTGATATT ACAAGGTTGGA
841 GCCCTGCTAC TATGACTGCT AACAAGAGAT CACCGATTCA ATCCAGAACA GCAGAGAATTG
901 ATGGAAGGTGA TTGGGAAAAT ATGTTGCAAA GAATTTTATAT TTATGATTGA CAAAATTTAC
961 CAAACTGCTG AGGTAAATGC CAAAGAGCTA AGATCATTAA ATTCGGCAC TTTTTATTAT
1021 AGAGTATAGA tataatcttag attaga
Figure 3 - Polypeptide sequence of P43 VP4. (RIX4414 VP4.pro; 775 aa)

1  MASLIYRQLL TNSYSVDLHD EIEQIGSEKT QNVTINPGPF AQTRYAPVNM DHGGSINDSTT
61  VEPILDGPYQ PTTFPPNDY WILINSNHTNG VYESTNNSD FWTAVVAIEP HVPVDRQYM
121  IFGESKQFNV SNDSNKKWKFL EMFRSSSQQE FYNRRRTLTS TRLVGIFKYG GRVWTIEGET
181  PRATTDSST ANLNNISITI HSEFYIIPRS QESKCNREYN NGLPPIQNTR NVPVLPLSSR
241  SIQYKRAQVN EDIVSKTSKL WKEMQYNRDI IIRFQFGNSI VKMGGLGKYKW SEISYKAANY
301  QYNYLDRGET VTAHTTCSVN GVNNFSYNGG PLPTDFGISR YEVIKENSYV YVDYWDDSSKA
361  FRNMYVYRSL AANLNSVKCT GGGYYFPSIPV GAWPVMNGGA VSLHFAQVTL STQPTDFVSL
421  NSLRFRFSILT VDEPFFSILR TRTVNLGLPL AANPNNONGY YEISRFSLSLI SLVPNTDDYQ
481  TPI tìmNSVTR QDLERQLTDL REEFNSLSEE IAMAQLIDLA LLPLDFMSMF SGIKSTIDLT
541  KSMATSVMKK FRKSKLATSI SEMTNSLSDA ASSASRNUSI RNSLISAISNW TNVSNDVSNV
601  TNSLNMDISTQ TISKKLRLE KEMITQTEGM SFDDISAAVL KTKIDMSTQI GKNLTDIVT
661  EASEKFIPKR SYRILKKDDEV MEINTEGRKFF AYKINTDFDEV PFVDNKFESL VIDSPVISAI
721  IDFKTLKNLN DNYGITRTEA LNLKSNPNM LRNFINGQNNP IIHRNRIEQLI LQCQL

Figure 4 - Polypeptide sequence of P43 VP7 (RIX4414 VP7.pro; 326 aa)

1  MYSIEYTTIL IFLISIIILN YILKSVTRIM DIYIYRSLLI YVALFALTRA QNYGLNLIPIT
61  GSMDTVYANS TQGFFLITST LCLYPTTEAS TQINDGEWKD SLSQMFILTG WPQSGYYKE
121  YSISVDFPSVD PQLYCDYLNVL KMKYDQMLEL DMSLADLIL NEWLCPNMFI TLYYQQSGE
181  SNSKWSMGSS CTVKVCPLNT QMLFQGCQTT NVDSFEVMVAE NEKLALDVVDV DGINHKINTL
241  TTCTCIRNCX KLGPRENAVQ IQVGGNVLQD ITATDPTTPQ TERNTRVNWK KWWQVFYTVTIV
301  DYINQIVQVM SKRSRSLNSA AFYYRV
Figure 5 - Polypeptide sequence of NSP4 protein of RIX4414 (175 aa).

1  MDKLADLNYT LSVITLMNDT LHSIIQDPGM AYPSYIASVL TVLFILHKAS IPTMKIALKT
61  SKCSYKVIKY CIVTIINLL KLAGYKEQVT TKDEIEEQMD RIVKEMRRLQ DMIDKLTTRE
121  IEQVELLKR1 HDNLITRPVD VIDMSKEFNFQ XNIKTLDEWE SGKNPYEPSE VTASIM

Figure 6 - Nucleotide sequence encoding NSP4 protein of RIX4414 (750 bp).

1  ggctttttt4aa agttctgttc cgagagagcg cgtcgggaaag gATGGATAAG CTTGCCGACC
61  TCAACTACAC ATGGAGTGTA ATCACITTTAA TGAATGACTAC ATTGCAATTCT ATAATTCAG
121  ATCTCTGTAAT GGCCTATTTT TCTATATATG CATCTGTTCT CACAGTTTTA TCTCATTAC
181  ATAAACGCTC AATTCCGAATG ATGGAAATAG CATTTGAAAATG ATCACAATGTT TCTATAAAG
241  TTGATAAATA TTGTATAGTC AGCTATTTTA ATACCTTCTT TAAAATGGCT GGATATAAAG
301  AGCCGATTAC TACAAAAAGC GAAAATGAGC AACCAGATGAA CAGAATTTT AAAGAGATGA
361  GACGTCAGCT GGATATATTG GTAATATATG TACTCCTGTA AATGGAAACAG GTTGAAATGC
421  TTTAACGTAT TACATGAAAC CTGATAACTA GACCAGTTGA CGTCATAGAT ATGGCAGAAG
481  AATTCAATCA GAAAAACTC AAAACCGTAG ATGAATGGGA GAGTGGAAAA AATCCATATG
541  AACCCTCAGA ATGACTGCA TCCATGTGAag aggttgtgatt accgctgctct gctctcggaa
601  gctggcggaa ctctccacgcc aagccccatt gacgttgtat gatgctgag aagccagct
661  caatctatgc gcgtgtgcct cagccttaat cccgtttaac caatccagcg agtggtaggac
721  gtaatggga ggaatggtct tagtgtgacc
Figure 7 - Polypeptide sequence of VP6 protein of RIX4414 (397 aa).

1 MEVLYSLSKTK LKDARDKIVE GTLYSNVSDL IQQFNQMIVT MNGNDFTQGG IGNLPVRNWT
61 FDPGLLGTTL LNLADANYVEN ARTTIEYFID PIONVCMDEM ARESQNRNGVS PQGSEALRKL
121 GIKFKRINFD NSSEYIENQ LQNRKRTGFG VFHKPNIFPP SFASSTLNNRSQ PSHDNLMGMTM
181 WLNAQGEIQV AGFDYSACIN APANIQQQEEH IVQLRRALMT ATITLLPDAE RFSFPRVINS
241 ADGAVLF1PN PVILRPVNVE VEFLLNGGII NTYQARFGTI IARNDFT1RL LFQLMRRPPNM
301 TPAYNLF1PQ AQPFQHHAVT GLLTRIESAV CESVLODANE TLLANV1AVR QEYAIPQ1GPV
361 FPPGNNWTEL ITNYSPSRED NLQRVFTV1AS IRSM1LIK

Figure 8 - Nucleotide sequence encoding VP6 protein of RIX4414 (1356 bp).

1 ggcttttaaaa cgaagtcttc gacATGGAGG TTCTGTACTC ACTGTCAAAA ACTCTTAAAG
61 ATGCTAGGGA CAAAATTTGT GTAAGGTACAT TATATTCTAA CGT1AGCGAT CTTATTCAGC
121 AATTTCACTAG ATATGAGTATG ACTT1CGGATT T1GGTCTATT AGGT1ACAACA CTTTTTCACT
241 TGATAGTCAT TTTATGGTAC GGAAGAA1GA GATCAATAGG ATATTTATTAT GATATTATTG
301 ATTAATGTATG TATGGATGAA AGT1CGAGAG AATCTCTAAG AAATGGAAGA TCGC1ACAAT
361 CTGAAGCCGT AGAAAGAGCTA GCCGGAATTA AATTTTATAG AATAATTTTG CATATTCATC
421 CAGAATACAT AGAAAGATGG AC1CTCACAAA ATAGAA1AGA GC1CGCCGGA TGGTTTTT
481 ATCAACCTTAA CTT1CGCTTCA TAC1CGGCTTT CATTATTCTT ATATAGACTT CAACCAAGTC
541 ATGATAATTTT AATGGGAACAC GT1GGGCGTAA ATG1CGGATG AGAAACA1TCA GTGCGCTGAT
601 TTGATTACCT ATGCGCCCTA AATGACCGAG CAGACACATCA GGT1AGCAGAT CGATATGTCT
661 ACGTCTAGGG CGAC1GCTACG ACAGCTACTA TCAC1ATTATG ACTGATACGA G1GAGATTGA
721 G1TTTCTCAGAG ATGATTAAGT CAC1GCTGTGT GC1CGAGACTG ATG1CGTCTCT AT1CGAGTTA
781 TTCTAAAGCC AAACAAAGTA AG1GGTACAG TTT1ATTGAA T1GACAAATT ATTAATACAT
841 AT1CGGCGTAG ATTTGTGACT ACTAGCGCAA GAAATTTTGA TACAATTTCT T1ATATTCTT
901 AGTTTGATCG G1C1GGCTAGT ATGAGCGAGG CTTTATG1GA ACTATTTCCA CAAGCA1AC
961 CT1TTTCTACGA CAC1GCTACG CT1GGACTTTA CATTATG1GA T1GATGCGAT GTGTGTGAAT
1021 CAGGCAGAGT G1G1CGCGGAC AAGGAGTTGT TAGGAAATGT G1GCGGCGGT GCGTCAAAGA
1081 AATGCGCATC AAGGCGCGAT GGA1ATTTGT AAGGAGTTGT TCCGAGGTT CACGGA1TTC
1141 AACTTTGCGG AATGGGAGAA GA1ACTTTGC AAG1CGGTTT CACGGTAAGT CTTATTAGGA
1201 GCATCTTGAT T1AGACTGGA ccaagctaat cattgtgctc caacatggtg ttgattattg
1261 gcattcatca gctattcaga ctcttcaagtt aagacatgta ttctatgcct gcattatgta
1321 gtaacctgtct gaatgatgta tgtgaggaggt tgtgacc
SEQUENCE LISTING

<110> GlaxoSmithKline Biologicals s.a.

<120> Vaccine

<130> VB61582

<160> 42

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 2350

<212> DNA

<213> Rotavirus

<400> 1

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gaataagac gaaagaaact cagaaattaa ctataaatcc ggtgtccttt 120
gcacagacta gatatgttcg cttcttgatt atattctatg ccaacctact atatact 180

gtgaatctta tttaactcct ttccttcgag cccatactaa atactcctcc tattgatat 240

tttggtgact cagctggtgc tattgaaacc cagctgtaac cagttagat aataatag 300

atatattggt ttagcaagca atttaatggt gatgactctg tctaaaatttg gcacagacta 360

gaaatgtttta gaagcactag tcacaaatgaa ttttataata gacgtcactt atcttctgat 420

acccagacta tttcattagt taataatggt ggaaggtgtt ggtggtccttc cattcagaat 480

gcgagagctca ccacatttca aatatcataa tattgtaatc gctaaaacc 540

ttgaaatagaa tcatgtataa atgctgattt atggtctttc tgaatcactc 600

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 660

tggaaatagaa tcatgtataa atgctgattt atggtctttc tgaatcactc 720

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 780

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 840

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 900

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 960

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 1020

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 1080

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 1140

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3120

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3180

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3240

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3300

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3360

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3420

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3540

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3600

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3660

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3720

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3780

tgatagatgt ttaataatggt ggaaggtgtt ggtggtccttc cattcagaat 1980

3840

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3900

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3960

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4020

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tagataaagg atagaattga acagttaata ctacaatgta ... tacaatgtaa attgtgagaa 2340
cgctattgag gatgtgacc 2359
<210> 2
<211> 1009
<212> DNA
<213> Rotavirus
<400> 3

<210> 2
<211> 1009
<212> DNA
<213> Rotavirus
<400> 3
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atatattaa aatcagtaac tcgaataatg gactacatta ... GIn Thr Arg Tyr Ala Pro VaI
3 5 4 0 4 5
Asn Trp Asp His GIy GIu lie Asn Asp Ser Thr Thr VaI GIu Pro lie
5 0 5 5 6 0
<210> 4
<211> 1046
<212> DNA
<213> Rotavirus

<400> 4
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tcagtaactc gaatattgga ctcactattattataggttcttgattttttctaatataa 180
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VaI 115 120 125
Asp Pro Gin Leu Tyr Cys Asp Tyr Asn Leu VaI Leu Met Lys Tyr
130 135 140
Asp Gin Asn Leu Glu Leu Asp Met Ser Glu Leu Ala Asp Leu H e Leu
145 150 155 160
Asn Glu Trp Leu Cys Asn Pro Met Asp H eThr Leu Tyr Tyr Tyr Gin
170 175
Gln Ser Gin Ser Asn Lys Trp H eSer Met Gin Ser Ser Cys Thr
180 185 190
VaI Lys VaI Cys Pro Leu Asn Thr Gin Met Leu Gin H eGin Cys Gin
195 200 205
Thr Thr Asn VaI Asp Ser Phe Glu Met VaI Ala Gin Asn Gin Lys Leu
210 215 220
Ala 11e VaI Asp VaI Asp Gin H eAsn His Lys H eAsn Leu Thr
225 230 235 240
Thr Thr Thr Cys Thr H eArg Asn Cys Lys Leu Gin Pro Arg Gin
245 250 255
Asn VaI Ala VaI H eGin VaI Gin Gin Gin Ser Asn VaI Leu H eThr
260 265 270
Ala Asp Pro Thr Thr Asn Pro Gin Thr Glu Arg Met Met Arg VaI Asn
275 280 285
Trp Lys Trp Trp Gin VaI Phe Tyr Thr H eVaI Asp Tyr H eAsn
290 295 300
Gln H e VaI Gin VaI Met Ser Lys Arg Ser Arg Ser Leu Asn Ser Ala
305 310 315 320
VaI Asn Gin Thr Tyr Tyr Thr Gin Met VaI Leu Thr Val Phe H eLeu His Lys
325 330 335

<210> 7
<211> 175
<212> PRT
<213> Rotavirus

<400> 7
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1 5 10 15
Met Asn Asp Thr Leu His Ser H eH eGin Asp Pro Gin Met Ala Tyr
20 25 30
Phe Ser Tyr H eAla Ser VaI Leu Thr VaI Leu Phe H eLeu His Lys
35 40 45
Ala Ser H ePro Thr Met Lys H eAla Leu Lys Thr Ser Lys Cys Ser
50 55 60
Tyr Lys VaI H eLys Tyr Cys H eVaI Thr H eH eAsn Thr Leu Leu
65 70 75 80
Lys Leu Ala Gin Tyr Lys Gin VaI Thr Lys Thr Lys Gin H eGin
85 90 95
Gln Gin Met Asp Arg H eVaI Lys Gin Gin Gin Met Met Gin Gin Leu Gin Met Met
100 105 110 115
H eAsp Lys Leu Thr Thr Arg Gin H eGin Gin VaI Gin Lys Leu Gin
120 125
Arg H eHis Asp Gin Leu H eThr Arg Pro Gin VaI Gin H eAsp Gin
130 135 140
Ser Lys Gin Lys Gin Asn H eLys Thr Lys Leu Gin Gin Thr Gin Gin
145 150 155 160
Ser Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin Gin
DNA Rotavirus

<210> 8
<211> 750
<212> DNA
<213> Rotavirus

<400> 8
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atcttggaat gcggtttaaa tcataatagc cagttggttc ctctttttt aacgattctt 180
ataaaagcttt aatctcaaccc atggaaatag cattgaaaaat atcttataag ctcttattac 240
tggactaata tggtaatagc cagtatttta aacggttgatt gcgtataaag 300
agcgacgttc cagaaagttc gcagaaatga cagaaatggaa gcagaaatggaa 360
gagtccagtc ggtatatttg tcatatatgg cagaaatggaa gcagaaatggaa 420
ttcgcatgta ctgttaacta cacgggcttt acacgctttt tcggagtcct 480
aatacattca gaaaaactatc aagcgtctta gcgcgtatttt gcgcgtatttt gcgcgtatttt 540
aacggtctga agtggtaattt aaacacggtct gcgcgtatttt gcgcgtatttt gcgcgtatttt 600
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gttatgga agaatggtctt tagttgacc 750

<210> 9
<211> 397
<212> PRT
<213> Rotavirus

<400> 9
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Lys lie VaI Glu Glu Thr Leu Tyr Ser Asn VaI Ser Asp Leu lie Gin
20 25 30
Gln Phe Asn Gin Met lie VaI Thr Met Asn Gln Asp Phe Gin Thr
35 40 45
Gl Glu lie Gln Asn Leu Pro VaI Arg Asn Trp Thr Phe Asp Phe Glu
50 55 60
Leu Leu Glu Thr Thr Leu Leu Leu Leu Asp Ala Asn Tyr VaI Gln Asn
65 70 75 80
Ala Arg Thr Thr lie Gln Tyr Phe lie Asp Phe lie Asp Asn VaI Cys
85 90 95
Met Asp Gln Met Ala Arg Gln Ser Gin Arg Asn Gln VaI Ser Pro Gin
100 105 110
Ser Gln Ala Leu Arg Lys Leu Ala Gln lie Lys Phe Lys Arg lie Asn
115 120 125
Phe Asp Asn Ser Ser Gln Tyr lie Gln Asn Trp Asn Leu Gin Asn Arg
130 135 140
Arg Gln Arg Thr Gln Phe VaI Phe His Lys Pro Asn lie Phe Pro Tyr
145 150 155 160
Ser Ala Ser Phe Thr Leu Asn Arg Ser Gin Pro Met His Asn Asn
165 170 175
Met Gln Thr Met Trp Leu Asn Ala Gln Ser Gln lie Gin VaI Ala Gln
180 185 190
Phe Asp Tyr Ser Cys Ala lie Asn Ala Pro Ala Asn lie Gin Gin Phe
195 200 205
Gln His lie VaI Gin Leu Arg Arg Ala Leu Thr Thr Ala Thr lie Thr
210 215 220
Leu Leu Pro Asp Ala GIu Arg Phe Ser Phe Pro Arg VaI H e Asn Ser
225 230 235 240
Ala Asp GIy Ala Thr Thr Trp Phe Phe Asn Pro VaI H e Leu Arg Pro
245 250 255
Asn Asn VaI GIu GIu VaI Phe Leu Leu Asn GIy Gin H e H e Asn Thr
260 265 270
Tyr GIy Ala Arg Phe GIy Thr H e H e Ala Arg Asn Phe Asp Thr H e
275 280 285
Arg Leu Leu Phe GIy Leu Met Arg Pro Pro Asn Met Thr Pro Ala VaI
290 295 300
Asn Ala Leu Phe Pro GIu Ala Gin Pro Phe Gin His His Ala Thr VaI
305 310 315 320
GIy Leu Thr Leu Arg H e GIu Ser Ala VaI Cys GIu Ser VaI Leu Ala
325 330 335
Asp Ala Asn GIu Thr Leu Leu Ala Asn VaI Thr Ala VaI Arg GIu Gin
340 345 350
Tyr Al H e Pro VaI GIy Pro VaI Phe Pro Pro GIy Met Asn Trp Thr
355 360 365
GIu Leu H e Thr Asn Tyr Ser Pro Ser Arg GIu Asp Asn Leu GIu Arg
370 375 380
VaI Phe Thr VaI Ala Ser H e Arg Ser Met Leu H e Lys
385 390 395

<210> 10
<211> 1356
<212> DNA
<213> Rotavirus

<400> 10
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aattcaatca aatgtagata actatgaatt gaaatgcct ccagctggga ggaaatgtga 180
atttactgg tagaaattgg acttctcagtt ttgctctatt agttacaaac tttcttggac 240
ttggatctaa ttttggatag aatggcaaga atccattgaa atatatttatt gatttattttg 300
ataaatgtatc tattggaagg atgccaagag atcctcggag aatctcgata tcgtacccaaat 360
cctgaagcttgt aaggaagaag gcggggttaa atattaagag aataatattc gataatttca 420
cagaatacga agaataatgg aaccttacaa atagagaca ggcacccgga ttggttttctc 480
atataaacctaa catttttctca taccctagct catttacatc aaatagactt caaccaatgc 540
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<210> 11
<211> 28
<212> DNA
Artificial Sequence

Oligonucleotide sharing homology with rotavirus nucleotide sequences

DNA Artificial Sequence

Oligonucleotide sharing homology with rotavirus nucleotide sequences

DNA Artificial Sequence

Oligonucleotide sharing homology with rotavirus nucleotide sequences

DNA Artificial Sequence

Oligonucleotide sharing homology with rotavirus nucleotide sequences

DNA Artificial Sequence
tgttgatatttt tctgtcgatc cac

Oligonucleotide sharing homology with rotavirus nucleotide sequences

ggttgctgag aagagaaat tagctatagt gg

Oligonucleotide sharing homology with rotavirus nucleotide sequences

ccactatagc taatttctca ttctcagaa cc

Oligonucleotide sharing homology with rotavirus nucleotide sequences

tggcttcgcc attttataga ca

Oligonucleotide sharing homology with rotavirus nucleotide sequences

atttcggacc atttataacc
<210> 20
<211> 22
<212> DNA
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<220>
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<400> 20
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<210> 21
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<220>
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<400> 21
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<210> 22
<211> 29
<212> DNA
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<220>
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<400> 22
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<210> 23
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
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<400> 23
tctattatttg tactttcata tactactcc 29

<210> 24
<211> 25
<212> DNA
<213> Artificial Sequence
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<400> 24
tcgatacagt ataagagagc acaag 25

<210> 24
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
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<400> 25
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<210> 25
<211> 27
<212> DNA
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<220>
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<400> 26
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<210> 26
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
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<400> 27
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<210> 27
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<210> 29
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<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 29
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<210> 30
<211> DNA
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<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 30
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<210> 31
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<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 31
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<210> 32
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<400> 32
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 33
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<210> 34
<211> 25
<212> DNA
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<220>
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<400> 34
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cgatcatatc gaatattaaa ggtat

<210> 35
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 35
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<210> 36
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 36
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<210> 37
<211> 32
<212> DNA
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<220>
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nucleotide sequences

<400> 37
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<210> 38
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 38
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<210> 39
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
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<400> 39
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<210> 40
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 40
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<210> 41
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 41
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<210> 42
<211> 25
<212> DNA
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<220>
<223> Oligonucleotide sharing homology with rotavirus nucleotide sequences

<400> 42
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