DRG® Norovirus Stool (EIA-4864)

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Please use only the valid version of the package insert provided with the kit.

INTENDED USE

The Norovirus ELISA is a 3rd generation enzyme immunoassay for the qualitative determination of the Norwalk viruses of Genogroups I and II in stool samples. *For in vitro use.*

SUMMARY AND EXPLANATION OF THE TEST

The name **Norovirus** stands for a group of viruses which are pathogenic to humans. These were first distinguished from the classical Caliciviruses as small round structured viruses (SRSVs) owing to their morphology. Caliciviruses were named as such because their typical surface structure resembled a string of cups (lat. calix = cup). Today, Noroviruses and the classical Caliciviruses are combined into one family, the Caliciviridae. All these SRS viruses have been named after the place where they were first isolated. Thus, the name Norwalk-like stood for all viruses which have been isolated during outbreaks of gastroenteritis and the name originated from the first time SRSV designated viruses were isolated in the city of Norwalk, Ohio, in the USA in 1972. The Norwalk virus was therefore the prototype of today's Noroviruses. Later, other isolates were named Snow Mountain agent, Hawaii agent and Montgomery county agent etc. in a similar way. According to the current directive of the "International Committee on Taxonomy of Viruses (ICTV)" of 2002, Caliciviruses are classified into the two human Genera, Norovirus and Sapovirus. There are also two genera which are pathogenic to animals. To what extent transmissions of animal Caliciviruses play a role in human infection is not currently known. All these viruses are icosahedral, single-stranded, RNA viruses, the capsid of which mainly consists of multiple copies of only one structural protein. None of these viruses can be cultured. They are the source of both isolated infections and outbreaks of gastroenteritis affecting all age groups. The genus, Norovirus, causes the majority of cases by far of all nonbacterial gastroenteritis.

According to current phylogenetic analyses, the Noroviruses are classified on three levels. Then, 29 genotypes (clusters) with numerous strains are divided into 5 genogroups. So far, human pathogens have only been described from Genogroup 1 (GGI) with 8 genotypes and from Genogroup 2 (GGI) with 17 genotypes. The remaining 4 genotypes are distributed between Genogroups 3 to 5. The symptoms of a gastroenteritis caused by Noroviruses are a strong feeling of nausea and severe vomiting and diarrhoea. The incubation period lasts between 6 and 48 hours with symptoms lasting for a further 12 - 60 hours afterwards in some cases. At only 100 virus particles, the infection dose is extremely small and is enough to satisfy the prerequisite for very effective spreading from humans to humans. Since the virus is eliminated both with the stool and with the vomit, airborne transmission due to the formation of aerosols containing the virus is also of importance in addition to faecal-oral transmission and manifests itself by the often very rapid spread in communal facilities. With a few exceptions, virus elimination only ceases after about two weeks and therefore also holds the risk of further spreading.

Re-infection may occur since comprehensive immunity is not possible, not least, because of the pronounced variability of Noroviruses. Apart from electron microscopy, most diagnostic methods carried out on the stool sample are limited to molecular genome determinations using PCR. Since these methods are relatively elaborate and require special expertise and laboratory equipment, there has been a need for a quick and simple screening test for a long time. With this ELISA and the now third generation further developed ELISA, this requirement has largely been taken into account. The Norovirus ELISA with monoclonal antibodies makes it possible to carry out very specific and highly sensitive determinations of Noroviruses from both genogroups. Unlike rotavirus infections, for example, the viral load in the stool with an acute Norovirus infection is 4 to 6 orders of magnitude lower. It is therefore particularly important to recover the stool specimens immediately the disease occurs.

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TEST PRINCIPLE

In the Norovirus ELISA, specific monoclonal antibodies are used in a sandwich type method. Specific antibodies against antigens of several different genotypes are applied to the surface of the well in the microwell plate. A suspension of the stool specimen to be examined and the controls, together with biotinylated monoclonal anti-Norovirus antibodies (Conjugate 1), are pipetted into the well in the microwell plate at ambient temperature (20 – 25 °C) for incubation. After a wash step, streptavidin peroxidase conjugate (Conjugate 2) is added and the microwell plate incubated at ambient temperature (20 – 25 °C). If Noroviruses are present in the stool specimen, a sandwich complex is formed made up of the immobilised antibodies, the Norovirus antigens and the antibodies conjugated with the biotin-streptavidin-peroxidase complex. Unattached streptavidin peroxidase conjugate is removed during a further washing phase. After adding the substrate, the attached enzyme changes the colour of the previously colourless solution in the wells of the microwell plate to blue if the test is positive. On adding the stop reagent, the colour changes from blue to yellow. The extinction is proportional to the concentration of Noroviruses present in the sample.

REAGENTS PROVIDED

There are enough reagents in the pack for 96 determinations.

PLATE	**96 det.**	**Microwell plate, 12 microwell strips (which can be divided) in the strip holder; coated with monoclonal antibodies against Noroviruses**
DILUENT 1	100 ml	Specimen-dilution buffer, protein-buffered NaCl solution; contains 0.1% NaN_3; ready for use; blue coloured
WASH	100 ml	Wash buffer, phosphate-buffered NaCl solution (10-fold concentrate); contains 0.1 % Thimerosal
CONTROL +	1.8 ml	Recombinant Norovirus antigens; ready for use; red coloured
CONJUGATE 1	10 ml	**Biotin-conjugated** antibodies against Noroviruses in stabilised protein solution; contains 0.05% Proclin 300; ready for use; blue coloured
CONJUGATE 2	10 ml	**Streptavidin peroxidase conjugate** in stabilised protein solution; contains 0.05% Proclin 300; ready for use; red coloured
SUBSTRATE	10 ml	Hydrogen peroxide/TMB; ready for use
STOP	6 ml	Stop reagent, 1 N sulphuric acid; ready for use

STORAGE INSTRUCTIONS

All reagents must be stored at 2 – 8 °C and can be used until the date printed on the label.

Providing the diluted wash buffer is stored at 2 – 8 °C, it can be used for a maximum of 4 weeks. Microbial contamination must be prevented. After the expiry date, the quality guarantee is no longer valid.

The aluminium bag must be opened with scissors in such a way that the clip seal is not torn off. Any microwell strips which are not required must be placed in the aluminium bag immediately and stored at 2 – 8 °C.

The colourless substrate must also be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

ADDITIONAL NECESSARY REAGENTS – AND NECESSARY EQUIPMENT

Reagents

- Distilled or deionised water

Accessories

- Test tubes
- Disposable pipettes (Article no: Z 0001)
- Vortex mixer (optional, see Section 9.3.)
- Micropipette for 50 -100 µl and 1 ml volume
- Measuring cylinder (1000 ml)
- Stop clock
- Washing unit for mictrotitration pipettes or multichannel pipettes (300 µl)
- Photometer for microwell plates (450 nm and reference filter ≥ 600 nm where necessary)
- Filter paper (laboratory towels)
- Waste container containing 0.5 % hypochlorite solution

PRECAUTIONS FOR USERS

For *in vitro* use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test must be strictly adhered to.

The positive controls in the kit contain inactivated Norovirus antigens. In spite of this, they and the patient samples must be treated as potentially infectious and handled in accordance with the national safety regulations.

Samples or reagents must not be pipetted by mouth and contact with injured skin or mucous membranes must be prevented. When handling the samples, wear disposable gloves and when the test is finished, wash your hands. Do not smoke, eat or drink in areas where samples or test reagents are being used.

The sample dilution buffer contains 0.1 % sodium azide as a preservative and conjugate and positive control contain 0.05 % Proclin 300 as a preservative. These substances must not be allowed to come into contact with the skin or mucous membrane.

The wash buffer contains 0.1 % Thimerosal as a preservative. Skin and mucous membranes must not be allowed to come into contact with this substance.

Urea peroxide can cause burns. Handle with care.

The stop reagent contains 1 N sulphuric acid. Avoid contact with the skin and clothing. If the skin is contaminated with the stop reagent, rinse it off with water.

All reagents and materials which have come into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121 °C for at least 1 hour.
CAUTION: To prevent the formation of poisonous gases, any liquid waste containing stop reagent must be neutralised before adding to hypochlorite solution.

SPECIMEN COLLECTION AND STORAGE

Stool specimens must be recovered as soon as possible within three days after the appearance of diarrhoea symptoms.

The test material must be stored at 2 – 8 °C until it is used in the test.
If the material is not to be used in the test within 3 days, we recommend that it be stored at -20 °C or colder.

Multiple freezing and thawing of the sample must be avoided.

Stool samples or rectal swabs should not be collected in transport containers which contain transport media with preservatives, animal sera, metal ions, oxidising agents or detergents since these may interfere with the Norovirus ELISA.

If rectal swabs have to be used, make sure that sufficient stool material (approx. 100 mg) is collected to carry out the test. During contact tracing, stool samples should also be taken from clinically inconspicuous contacts in order to identify asymptomatic carriers.

TEST PROCEDURE

General information

All reagents and the microwell plate must be brought to room temperature (20 – 25 °C) before use. The microwell strips must not be removed from the aluminium bag until they have reached room temperature.

The reagents must be thoroughly mixed immediately before use.

After use, the microwell strips (in sealed bags) and the reagents must be stored at 2 – 8 °C. Once used, the microwell strips must not be used again.

The reagents and microwell strips must not be used if the packaging is damaged or the vials are leaking.

When dropping the reagent, the vial should be held vertically.

Both the specimen suspension and the reagents should be dropped or pipetted into the microwells without touching the edge.

In order to prevent cross contamination, the specimens must be prevented from coming into direct contact with the kit components and the test must not be carried out in direct sunlight.

We recommend that the microwell plate is covered or sealed with film in order to prevent evaporation losses.

Preparing the wash buffer

1 part wash buffer concentrate is mixed with 9 parts distilled water.
Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37 °C.

Preparing the samples

Place 1 ml sample dilution buffer Diluent 1 in a labelled test tube.

Suck up liquid stool into a disposable pipette (Article no Z 0001) until it rises to just above the second mark (approx. 100 µl) and suspend it in the buffer which was placed in the tube beforehand.

With solid stools, take an equivalent amount (about 50 - 100 mg) with a spatula or a disposable inoculation loop and suspend it in solution. Homogenise the stool suspension either by suction and ejection from a disposable pipette or, alternatively, by mixing in a vortex mixer. After leaving the suspension to stand for a short period (10 minutes) in order to allow the coarse stool particles to settle, the resulting supernatant of the stool suspension can be used directly in the test.

If the test is carried out in an ELISA machine, this supernatant **must** be free of particles. In this case, it is advisable to centrifuge the specimen at 5000 rpm (approx. 2300 – 2500 G) for 5 minutes.

First incubation

After selecting a sufficient number of wells in the frame, pipette 2 drops (or 100 µl) of Positive Control, the specimen-dilution buffer (Diluent 1) (= negative control) or the stool suspension in the wells.

Then add 2 drops (100 µl) of the biotin-conjugated antibody (Conjugate 1) and, after mixing thoroughly (by lightly tapping on the edge of the plate),
incubate at room temperature (20 – 25 °C) for 60 minutes.

Washing

Careful washing is important in order to achieve the correct results and should therefore take place strictly according to the instructions. The incubated substance in the wells must be emptied into a waste container containing hypochlorite for disinfection. After this, knock out the plate onto absorbent paper in order to remove the residual moisture. After this, wash the plate 5 times using 300 µl wash buffer each time. Make sure that the wells are emptied completely by knocking them out after each wash on a part of the absorbent paper which is still dry and unused.

Note: When using a washing machine, make sure that the machine is correctly adjusted to the type of microwell plate being used. Furthermore, a stool suspension which is not completely particle-free before the first wash should be removed manually by centrifuging it from the cavities in order to avoid blocking the wash needles. Also make sure that all of the liquid is sucked away during each washing phase. After washing for the last time, knock out the plate thoroughly onto clean absorbent paper or a laboratory towel in order to remove any residual moisture. In order to achieve the optimum washing results, it is advisable to wash in overflow mode with at least 600 µl wash buffer per well and wash step. The number of wash steps can be increased to more than 5 and this can occasionally lead to better washing results.

Second incubation

Add 2 drops (100 µl) of the streptavidin-peroxidase conjugate (Conjugate 2) to the wells and
incubate at room temperature (20 – 25 °C) for 30 minutes.

Washing

See Section 9.5.

Third incubation

Add 2 drops (100 µl) of Substrate to each well.

Then incubate the plate at room temperature (20 - 25 °C) for 15 minutes in the dark.

After this, stop the reaction by adding 1 drop (50 µl) Stop Solution to each well.
After mixing carefully (by lightly tapping the side of the plate) measure the extinction at 450 nm using a reference wavelength ≥ 600 nm (optional). Then calibrate the zero against air i.e. without a microwell plate.

Note: High positive patient samples may cause the substrate to precipitate as a black sediment.

QUALITY CONTROL – INDICATIONS OF REAGENT EXPIRY

For quality control purposes, positive and negative controls must be used each time the test is carried out in order to make sure that the test has been carried out correctly and the reagents are stable.

The test has been carried out correctly if the extinction (O.D.) for the negative control is less than 0.2 at 450 nm and the measured value for the positive control is greater than 0.5 at 450 nm.

If the value is greater than 0.2 for the negative control, this may indicate that there was insufficient washing.

If the values differ from those required or if the substrate is turbid or has turned blue before adding to the wells, this may be an indication that the reagents have expired.

If the stipulated values are not met, the following points must be checked before repeating the test:

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned blue must not be used.

If the conditions are still not fulfilled after repeating the test, please consult the manufacturer or your local distributor.

EVALUATION AND INTERPRETATION

Calculating the cut-off

In order to establish the cut-off, 0.15 extinction units are added to the measured extinction for the negative control.

Cut-off = Extinction for the negative control + 0.15

Test result

Samples are considered **positive** if their extinction is more than 10 % above the calculated cut-off.

Samples are considered **equivocal** and must be repeated if their extinction is within ± 10 % of the cut-off.
If repeating the test with a fresh stool sample again yields a value in the grey range, the sample must be considered negative.

Samples with extinctions more than 10 % below the calculated cut-off must be considered **negative**.

LIMITATIONS OF THE METHOD

The Norovirus ELISA determines Norovirus antigens of the two human pathogenic genogroups 1 and 2 in stool specimens. Although not all the genotypes with the associated subtypes have been tested, almost all the genotypes which are known so far from outbreaks of gastroenteritis are recorded where the viral load at the time of recovering the specimen is not below the ELISA detection limit. The test cannot be used to derive a relationship between the determined extinction and the occurrence of serious clinical symptoms. **The results obtained must always be interpreted in combination with the clinical picture.** Asymptomatic virus carriers also exist (contact tracing). Asymptomatic virus carriers also exist (contact tracing) which can be recorded using ELISA. The viral load at the time of recovering the specimen is crucial in each case.

A **positive** result does not rule out the presence of another infectious pathogen. Double and multiple infections or potential gastroenteritis pathogens are adequately described and can be determined by differential diagnostics. The clinical symptoms are much more pronounced in these cases than in monocausal cases.

A **negative** result does not necessarily mean that there is no Norovirus infection. This can be caused by intermittent excretion of the virus, by recovering the specimen at an unsuitable time (see Point 8 "Specimen collection and storage"), by the virus load being too small or by not handling the specimen correctly. If the patient is suspected to have a Norovirus infection, another stool sample should be tested.

An **equivocal** result may be caused by non-homogeneous distribution of the virus in the stool specimen, by the virus load being equivocal for the ELISA, by the infection subsiding or by inadequately washed microwells. If this happens, a second suspension should be tested from the same sample or a second stool sample collected for the test.

Meconium specimens have so far not been validated with Norovirus ELISA and must therefore be interpreted with care.

Tests using Norovirus-negative stool samples which have been spiked with various baby-care products such as creams, oils and ointments which can get into the specimen when recovering the stool have so far been found to be unaffected by these agents. Neither were Norovirus positive stool samples impaired by the market products tested.

PERFORMANCE CHARACTERISTICS

Test quality

In a validation study with the Norovirus ELISA, 3rd Generation, 60 specimens which had been recovered during a routine diagnostic study were analysed during the winter of 2004/2005 at the Institute of Virology of the University of Dresden using RT-PCR and then kept frozen (-20 °C) for later use. A further 123 specimens from the winter season of 2006 (January to May) were used which were also kept frozen for later use after RT-PCR determination. The results of both specimen panels are listed in Table 1.

Table 1: Correlation of the Norovirus ELISA 3rd Generation with the RT-PCR

		Norovirus ELISA Panel WS 2004/5		Norovirus ELISA Panel WS 2006/5	
		+	-	+	-
RT-PCR	+	28	2	55	15
	-	0	30	0	53
Sensitivity:		93.3 %		78.6 %	
Specificity:		100.0 %		100.0 %	
PPV:		100.0 %		100.0 %	
NPV:		93.8 %		77.9 %	
Accuracy:		96.7 %		87.8 %	

Cross reactivity

Different pathogenic organisms of the intestinal tract have been tested using the Norovirus ELISA and have shown no cross reactivity. The tests were carried out on undiluted bacterial or viral suspensions with organism concentrations ranging from 10^6 to 10^9 organisms per ml. The results are listed in Table 2.

Table 2: Cross reactions with pathogenic micro-organisms

Serial no:	Test germ	Origin	OD = 450 mm
1	Acinetobacter Iwoffii	Culture	0.044
2	Aeromonas hydrophila anaerogenes	Culture	0.043
3	Aeromonas hydrophila hydrophila	Culture	0.043
4	Citrobacter freundii	Culture	0.042
5	Citrobacter freundii	Culture	0.042
6	Enterobacter cloacae	Culture	0.044
7	Enterococcus faecalis	Culture	0.041
8	Enterococcus faecium	Culture	0.079

Serial no:	Test germ	Origin	OD = 450 mm
9	E. coli	Isolate	0.041
10	E. coli	Isolate	0.043
11	E. coli	Isolate	0.043
12	E. hermannii	Culture	0.042
13	Lactococcus lactis	Culture	0.043
14	Listeria innocua	Culture	0.042
15	Proteus mirabilis	Culture	0.042
16	Proteus mirabilis	Culture	0.041
17	Proteus vulgaris	Culture	0.042
18	Providencia stuartii	Culture	0.043
19	Pseudomonas aeroginosa	Culture	0.047
20	Pseudomonas fluorescens	Culture	0.038
21	Pseudomonas fluorescens	Culture	0.055
22	Pseudomonas putida	Culture	0.04
23	Salmonella Agona	Isolate	0.038
24	Salmonella choleraesuis	Culture	0.039
25	Salmonella infantis	Isolate	0.04
26	Salmonella Ohio	Isolate	0.041
27	Salmonella typhimurium	Isolate	0.038
28	Serratia proteamaculans (liquefaciens)	Culture	0.039
29	Shigella flexneri	Culture	0.039
30	Shigella sonnei	Culture	0.075
31	Staphylococcus aureus	Culture	0.038
32	Streptococcus agalactiae	Isolate	0.041
33	Streptococcus dysgalactiae	Isolate	0.038
34	Streptococcus uberis	Isolate	0.04
35	E. coli (O157:H-)	Isolate	0.042
36	E. coli (O116:H21)	Isolate	0.042
37	E. coli (O111:H-)	Isolate	0.045
38	E. coli (O22:H8)	Isolate	0.045
39	E. coli (O26:H11)	Isolate	0.096

Serial no:	Test germ	Origin	OD = 450 mm
40	Candida albicans	Culture	0.043
41	Salmonella enteritidis	Culture	0.043
42	Campylobacter jejuni	Culture	0.044
43	Campylobacter coli	Culture	0.046
44	Campylobacter fetus	Culture	0.045
45	Helicobacter pylori	Culture	0.044
46	Morganella morganii	Culture	0.043
47	Astrovirus Zellkulturüberstand $>10^7$	Culture	0.089
48	Astrovirus Probe	Stool 1:10	0.047
49	Adenovirus Zellkulturüberstand $>10^7$	Culture	0.043
50	Adenovirus Probe	Stool 1:10	0.047
51	Rotavirus Zellkulturüberstand $>10^9$	Culture	0.087
52	Rotavirus Probe	Stool 1:10	0.048
53	H.pylori Probe	Stool 1:10	0.054
54	C.perfringens 50 µg/ml	Toxoid	0.09
55	Shigatoxin STX1	Toxoid	0.045
56	Shigatoxin STX2	Toxoid	0.079
57	Cl.sordellii	Culture	0.045
58	Cl.difficile	Culture	0.047
59	Cryptosporidium parvum $>10^7$	Culture	0.089
60	Campylobacter Probe	Stool 1:10	0.064
61	Giardia Lamblia Probe	Stool 1:10	0.048
62	Ent. Histolytica	Stool 1:10	0.08
63	Salmonella enteritidis Probe	Stool 1:10	0.075

Precision

The intra-assay reproducibility was determined from a 24-fold batch of negative controls, specimens containing high (HPR), average (MPR) and a weakly positive (LPR) Genogroup I capsid stool sample and specimens containing Genogroup II capsids. The average of the extinction values as well as the standard deviations and coefficients of variance are summarised in Table 3.

Table 3: Intra-assay reproducibility

Genogroup I (GG1 /1 ; GG1 /3)				
	HPR	**MPR**	**LPR**	**NC**
MV (OD)	2.116	1.134	0.521	0.046
SD	0.01	0.08	0.07	0.01
CV %	3.8	6.7	3.4	10.8
Genogroup II (GG2 /1 ; GG2 /2 , GG2 / 3)				
	HPR	**MPR**	**LPR**	**NC**
MV (OD)	2.128	1.160	0.538	0.045
SD	0.073	0.076	0.04	0.01
CV %	3.4	6.6	6.7	10.9

The inter-assay reproducibility of the Norovirus ELISA was determined from a 9fold lot of positive and negative controls and a high (HPR), average (MPR) and a weakly positive (LPR) specimen on 3 consecutive days and with 3 different test kits from one lot. The average of the measured extinction values as well as the standard deviations and coefficients of variance are summarised in Table 4.

Table 4: Inter-assay reproducibility

	PK	HPR	MPR	LPR	NC
MV (OD)	2.529	2.127	1.111	0.505	0.045
SD	0.01	0.03	0.02	0.01	0.001
CV %	0.4	1.4	1.8	1.0	3.4

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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