Product Description
SALSA® MLPA® Probemix P083-D2 CDH1

To be used with the MLPA General Protocol.

Version D2
For complete product history see page 9.

Catalogue numbers:
- **P083-025R:** SALSA MLPA Probemix P083 CDH1, 25 reactions.
- **P083-050R:** SALSA MLPA Probemix P083 CDH1, 50 reactions.
- **P083-100R:** SALSA MLPA Probemix P083 CDH1, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

Intended purpose
The SALSA MLPA Probemix P083 CDH1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *CDH1* gene in genomic DNA isolated from human peripheral whole blood specimens and Research Use Only (RUO) assay on DNA isolated from fresh and frozen tumour tissue. P083 CDH1 is intended to confirm a potential cause for and clinical diagnosis of Hereditary Diffuse Gastric Cancer and/or Lobular Breast Cancer, and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P083 CDH1 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *CDH1* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations from DNA extracted from formalin-fixed paraffin embedded (FFPE) tumour materials.

[1] Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
Germline heterozygous mutations in the *CDH1* gene have been reported in approximately 30-50% of families with a hereditary predisposition to diffuse gastric cancer (Oliveira *et al*. 2013). Cadherin-1 (CDH1) also known

as E-cadherin is a classical cadherin from the cadherin superfamily. The encoded protein is a calcium dependent cell-cell adhesion glycoprotein. Reduced expression of *CDH1* is regarded as one of the main molecular events involved in dysfunction of the cell-cell adhesion system, triggering cancer invasion and metastasis.

Hereditary diffuse gastric cancer (HDGC) accounts for <1% of all gastric cancer patients (Sugimoto *et al*. 2015). The majority of the cancers in individuals with a *CDH1* pathogenic variant occur before the age of 40. The penetrance of HDGC is incomplete and the estimated cumulative risk of gastric cancer by age 80 years is 70% for men and 56% for women. The frequency of *CDH1* large deletions in hereditary diffuse gastric cancer is ~4% (Sugimoto *et al*. 2015, Oliveira *et al*. 2013, https://www.ncbi.nlm.nih.gov/books/NBK1139/).

If gastric cancer is detected early and is resected, the 5-year survival rate can be greater than 90%. However, HDGC has an infiltrative growth pattern and is difficult to diagnose. Once symptoms appear, affected individuals are in an advanced stage of the disease with a poor prognosis, with a 5-year survival rate lower than 20% (Oliveira *et al*. 2013). Therefore, clinical management options for carriers of germline *CDH1* mutations include prophylactic gastrectomy and/or an intensive regimen of endoscopic surveillance. However, the value of a surveillance regime is not yet proven, as in most cases the gastric cancer is not detected until it reaches an incurable, advanced stage.

In addition, women also have a 42% risk for developing lobular breast cancer. For women carrying a *CDH1* mutation, regular breast screening is recommended (Oliveira *et al*. 2013). Lobular breast cancer can be the first manifestation of HDGC, also in patients without a history of gastric cancer (Benusiglio *et al*. 2013).

More information on HDGC is available at: https://www.ncbi.nlm.nih.gov/books/NBK1139/.

Gene structure
The *CDH1* gene spans ~98 kilobases (kb) on chromosome 16q22.1 and contains 16 exons. The *CDH1* LRG_301 is available at www.lrg-sequence.org and is identical to GenBank NG_008021.1.

Transcript variants
For *CDH1*, multiple variants have been described. Transcript variant 1 encodes the longest isoform (1) (NM_004360.5; 4811 nucleotides (nt); coding sequence (CDS) 125-2773; https://www.ncbi.nlm.nih.gov/gene/999). The ATG translation start site is located in exon 1 and the stop codon is located in exon 16.

Exon numbering
The *CDH1* exon numbering used in this P083-D2 CDH1 product description is the exon numbering from the LRG_301 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P083-D2 CDH1 contains 35 MLPA probes with amplification products between 130 and 400 nt. This includes 20 probes for the *CDH1* gene region, and one upstream flanking probe and one downstream flanking probe. In addition, 13 reference probes are included that detect autosomal chromosomal locations with relatively copy number stable regions in various cancer types including colorectal cancer. Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten *et al*. 2002) are described in the MLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA from peripheral blood, free from impurities known to affect MLPA reactions. In a research setting only, DNA extracted from fresh or frozen tumour tissue can be used. Never use DNA isolated from FFPE tissue samples. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different healthy individuals without a (family) history of diffuse gastric cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA12074 and NA19092 from the Coriell Institute have been tested at MRC Holland and can be used as positive control samples to detect a deletion of the whole *CDH1* gene (the flanking probes for *ADGRG1* and *PLCG2* are not affected) and a multi-exon duplication of *CDH1* (from exon 4 to the downstream region of this gene), respectively. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics
The frequency of *CDH1* large deletions in hereditary diffuse gastric cancer is ~4% (Sugimoto *et al*. 2015; Oliveira *et al*. 2013; https://www.ncbi.nlm.nih.gov/books/NBK1139/). The analytical sensitivity and specificity for the detection of deletions or duplications in the *CDH1* gene is very high and can be considered >99% (based on a 2007-2021 literature review and https://www.ncbi.nlm.nih.gov/gtr/).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis

Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The expected results for *CDH1* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 0 (homozygous deletion), 3 (heterozygous duplication) or 4 (homozygous duplication). A homozygous deletion (copy number 0) of the *CDH1* gene cannot be expected in blood derived DNA, because such a deletion is associated with embryonic lethality. However, in tumour tissue it can occur. The standard deviation of each individual probe over all the reference samples should be ≤0.10. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic or subclonal cases.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga *et al*. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz *et al*. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.

- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *CDH1* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P083 CDH1.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- When used on tumour DNA (for Research Use Only): MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample.
- Use of fresh/frozen tumour tissues (for Research Use Only) can result in lower quality of the extracted DNA due to deviations in preanalytical steps (e.g. sample storage conditions). This might result in higher probe standard deviation. Warnings during the Fragment Analysis using Coffalyser.Net will indicate that the MLPA experiment was not optimal on the specific sample(s) used.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***CDH1* mutation database**

The LOVD *CDH1* page can be found at https://databases.lovd.nl/shared/genes/CDH1. We strongly encourage users to deposit positive results in the LOVD. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *CDH1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P083-D2 CDH1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*CDH1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 19616-L26704	4p13	
138	**CDH1 probe** 20903-L28968		**Upstream**
142	Reference probe 10679-L11261	6p12	
148	**CDH1 probe** 12653-L19725		**Exon 8**
154 ¬	ADGRG1 probe 10195-L10655		Centromeric
160	**CDH1 probe** 02409-L19374		**Exon 7**
166	**CDH1 probe** 20905-L30071		**Exon 9**
172	**CDH1 probe** 20904-L29487		**Downstream**
181	**CDH1 probe** 21392-L01862		**Exon 16**
186	Reference probe 06587-L30000	2q24	
193	**CDH1 probe** 12654-L19369		**Exon 2**
202	**CDH1 probe** 20906-L28971		**Exon 10**
208	Reference probe 11611-L12371	12p13	
216 ¬	PLCG2 probe 18052-L29486		Telomeric
225	Reference probe 10244-L15878	10p12	
232	**CDH1 probe** 20907-L29534		**Exon 11**
242 ¥ Δ	**CDH1 probe** 20908-L32322		**Exon 1**
250	Reference probe 14971-L29482	6q22	
260	**CDH1 probe** 20909-L28974		**Exon 3**
265	**CDH1 probe** 02413-L19371		**Exon 13**
274	Reference probe 17873-L22132	2p21	
283	**CDH1 probe** 02414-L01860		**Exon 14**
292	Reference probe 08722-L28962	9q21	
301	**CDH1 probe** 02407-L01853		**Exon 5**
310	Reference probe 09065-L09234	19p13	
316	**CDH1 probe** 02415-L19372		**Exon 15**
329	**CDH1 probe** 02408-L13240		**Exon 6**
337	**CDH1 probe** 20910-L28975		**Exon 16**
348	Reference probe 08802-L26551	2p13	
357	Reference probe 03092-L30095	11p13	
364	**CDH1 probe** 12656-L19936		**Exon 4**
372	Reference probe 06016-L21128	19q13	
382	**CDH1 probe** 16885-L19718		**Exon 12**
391	**CDH1 probe** 12657-L14803		**Exon 2**
400	Reference probe 09770-L28964	15q21	

[a] See section Exon numbering on page 2 for more information.

¥ Changed in version D2. Minor alteration, no change in sequence detected.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. *CDH1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*CDH1* exon[a]	Location / Ligation site NM_004360.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
154 ¬	10195-L10655	*ADGRG1* gene	16q13	GATTGTGGTACA-GAACACCAAAGT	11 **M**b
138	20903-L28968	Upstream	3.1 kb before exon 1	AGTGGGAAAGGA-AATACCGGAGCC	3.1 kb
242 Δ	20908-L32322	Exon 1	36-37	CTGTGAGCTTGC-GGAAGTCAGTTC	0.6 kb
		start codon	*125-127 (Exon 1)*		
391	12657-L14803	Exon 2	369 nt before exon 2	CCGGGGATAAGA-AAGTGAGGTCGG	0.4 kb
193	12654-L19369	Exon 2	234-235	CGCCGAGAGCTA-CACGTTCACGGT	63.4 kb
260	20909-L28974	Exon 3	395-396	GGCCTCTACGGT-TTCATAACCCAC	6.7 kb
364	12656-L19936	Exon 4	581-582	TCAGAAGACAGA-AGAGAGACTGGG	0.2 kb
301	02407-L01853	Exon 5	695-696	AGGTTTTCTACA-GCATCACTGGCC	1.5 kb
329	02408-L13240	Exon 6	844-845	TCCAACGGGAAT-GCAGTTGAGGAT	1.5 kb
160	02409-L19374	Exon 7	997-998	GACGCGGACGAT-GATGTGAACACC	0.4 kb
148	12653-L19725	Exon 8	1161-1162	CCTGGTGGTTCA-AGCTGCTGACCT	1.2 kb
166	20905-L30071	Exon 9	1317-1316 reverse	CAGCATCAGTCA-CTTTCAGTGTGG	2.3 kb
202	20906-L28971	Exon 10	1583-1584	CCCCCATCTTTG-TGCCTCCTGAAA	3.7 kb
232	20907-L29534	Exon 11	1719-1720	CACTGCCAACTG-GCTGGAGATTAA	2.9 kb
382	16885-L19718	Exon 12	2052-2053	CATTCAGTACAA-CGACCCAAGTGG	1.3 kb
265	02413-L19371	Exon 13	2166-2167	AGTGACCACCTT-AGAGGTCAGCGT	4.7 kb
283	02414-L01860	Exon 14	2318-2319	TGCTGTTTCTTC-GGAGGAGAGCGG	1.5 kb
316	02415-L19372	Exon 15	2479-2480	GTGACTCGTAAC-GACGTTGCACCA	3.7 kb
181	21392-L01862	Exon 16	2654-2655	CCGAAGCTGCTA-GTCTGAGCTCCC	1.6 kb
		stop codon	*2771-2773 (Exon 16)*		
337	20910-L28975	Exon 16	4209-4210	AGGTGGGTCTAC-CTCATCTCTGAA	1.1 kb
172	20904-L29487	Downstream	469 nt after exon 16, reverse	AAAGTTACCATG-TGAATGTAGTTT	13 **M**b
216 ¬	18052-L29486	*PLCG2* gene	16q23.2	TTCCAAGGGGGT-GGGAGCATGACT	-

[a] See section Exon numbering on page 2 for more information.

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
274	17873-L22132	*PPM1B*	2p21	AGCAGAAAATCA-TTAGCATTTCCC	02-044,313
348	08802-L26551	*DYSF*	2p13	CTGTGGGCTGCT-TCTTGGTGTCCA	02-071,583
186	06587-L30000	*SCN2A*	2q24	TGGTCAACAACT-ACAGTGAGTGCA	02-165,939
130	19616-L26704	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
142	10679-L11261	*PKHD1*	6p12	ATTTGCGAGGAA-AGTTCCCAATGC	06-051,990
250	14971-L29482	*LAMA2*	6q22	GCACCACCTAGG-AGAAAACGAAGG	06-129,844
292	08722-L28962	*PCSK5*	9q21	GTGCAGAGCTGT-AGTATCAGCTAT	09-077,993
225	10244-L15878	*NEBL*	10p12	CTGGGATCCTTT-TCTGTTCACTCA	10-021,226
357	03092-L30095	*PAX6*	11p13	AGTCATATTCCT-ATCAGCAGTAGT	11-031,771
208	11611-L12371	*KCNA1*	12p13	GAGAGTGCTGTT-TATCGTCATTTG	12-004,889

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
400	09770-L28964	*SPG11*	15q21	GAGCTGATACCA-GCATTGGATTTA	15-042,708
310	09065-L09234	*CACNA1A*	19p13	CTCAGGCCTTCT-ACTGGACTGTAC	19-013,288
372	06016-L21128	*PRPF31*	19q13	ACAAGTGCAAGA-ACAATGAGAACC	19-059,318

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

P378 MUTYH Includes probes for all exons of MUTYH, which is involved in development of colon and stomach cancer.

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P083 product history	
Version	*Modification*
D2	The CDH1 exon 1 probe has been modified, and two reference probes have been removed.
D1	Five CDH1 probes have been replaced, three have been added, and two new flanking probes have been included. Seven reference probes have been replaced and two have been removed. Five probes have a small change in length but not in the sequence detected.
C2	Five reference probes have been replaced.
C1	Two CDH1 probes and several reference probes have been replaced/added. In addition, the 88 and 96 nt control fragments have been replaced (QDX2).
B1	Five CDH1 probes and six reference probes have been replaced.
A2	Extra control fragments at 88-96-100-105 nt have been added and five probes have a slightly different length and/or peak height. No change in sequences detected.

Implemented changes in the product description

Version D2-04 – 21 March 2024 (04P)
- NA19092 added to Positive control sample section.
- Clinical background updated to reflect most recent literature.
- Older selected publications with small cohorts removed.

Version D2-03 – 24 July 2023 (04P)
- Product is no longer registered as IVD in Morocco.

Version D2-02 – 15 February 2022 (04P)
- Product description rewritten and adapted to a new template.
- Various minor textual or layout changes.
- Ligation sites of the probes targeting the *CDH1* gene updated according to new version of the NM_ reference sequence.
- *References* and *Selected Publications* were curated and new literature was included.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version D2-01 — 27 October 2020 (02P)
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Intended use changed to Intended purpose using new template text.
- Various minor textual or layout changes.
- Gene name of flanking probe 10195-L10655 was updated to ADGRG1.

Version D1-03 – 16 March 2020 (02P)
- Product description restructured and adapted to a new template.
- New warning added to Table 1 and 2 for variability of probe 20908-L29130.
- New selected publications were added.
- Colombia was added as country with IVD status.

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*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.