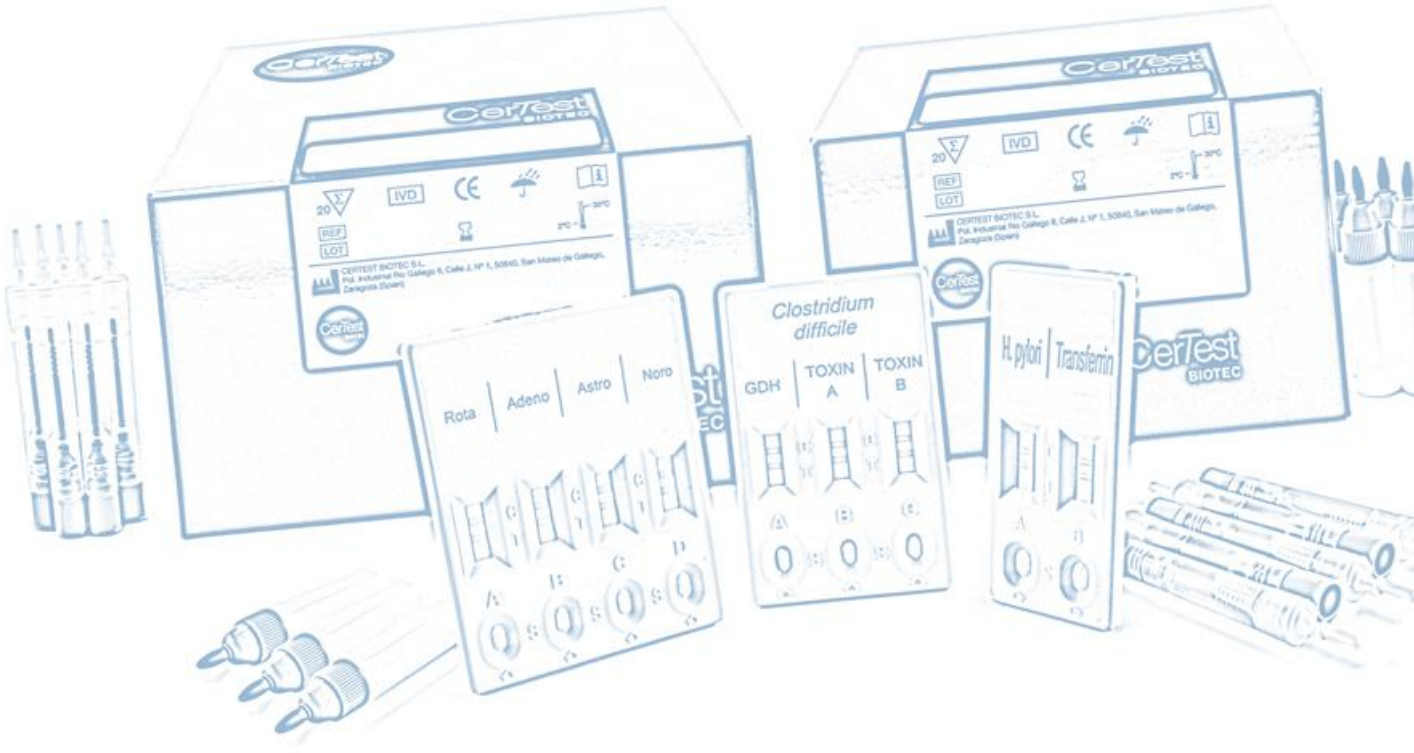
CerTest BIOTEC

EVALUATION OF

CERTEST *Clostridium difficile* antigen GDH TEST vs Viasure *Clostridium difficile* Real Time Detection kit



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1. LOCATION

Evaluation made in our facilities (CerTest Biotec S.L., Pol. Industrial Río Gállego II, Calle J, nº1, 50840 San Mateo de Gállego, Zaragoza, (Spain)) from 17th April to 2nd June 2017 for *Clostridium difficile* antigen GDH evaluation with fecal samples from Hospital.

- Hospital Lozano Blesa (Zaragoza, Zaragoza, SPAIN)

2. AIM

To determine the performance characteristics of a rapid immunocromatographic test, CerTest *Clostridium difficile* antigen GDH (CerTest Biotec S.L, Spain) an evaluation, with fecal samples, was performed comparing the results obtained by an immunochromatographic test (CerTest *Clostridium difficile* antigen GDH CerTest) and qPCR technology. qPCR has been considered as gold standard for this evaluation.

3. RESPONSABILITIES

Evaluation performed by CerTest laboratory technicians:

Marisa Sanchez	Laboratory technicians
Sandra García	

Final approval of the Immunochromatographic Product Manager: **Manuel Villacampa.**

4. SAMPLE

The stool samples were prepared from patients collected by Hospital:

Hospital Lozano Blesa.

These patients showed some symptoms that could be produced by *Clostridium difficile* antigen GDH.

The collected samples were stored at -20ºC (frozen samples) till tested following the kit instructions for use of every test.

The samples have been evaluated by qPCR technique and by all lateral flow tests studied.

5. ABBREVIATIONS AND DEFINITIONS

Abbreviations:

++ & +++: very strong positive results	**LF:** Lateral Flow
+: positive result	**IFU:** instructions for use
±: for low intensity positive result	**CI:** confidence interval
-: for negative result	**PPV:** positive predictive value
Min: minute	**NPV:** negative predictive value
Not ev.: not evaluated	**Cq**: Threshold Cycle (Ct)

Definitions:

Sensitivity: It is the ability of a test to correctly classify an individual as “diseased”.

Specificity: It is the ability of a test to correctly classify an individual as *disease-free*

PPV: It is the percentage of patients with a positive test who actually have the disease.

NPV: It is the percentage of patients with a negative test who do not have the disease.

Kappa Value: the measure of concordance between reference assay and evaluated test was calculated using Cohen´s Kappa coefficient. The interpretation should be as follow:

Kappa values (IK)>0.4 → weak concordance

Kappa values (IK) between 0.4 to 0.6 → high concordance

Kappa values (IK) > 0.6 → excellent concordance

Confidence interval: is a type of interval estimate of a population parameter. It is an observed interval (i.e., it is calculated from the observations), in principle

different from sample to sample, that potentially includes the unobservable true parameter of interest. How frequently the observed interval contains the true parameter if the experiment is repeated is called the confidence level.

Lateral Flow: Diagnostic Detection Immunological Technology based on the capture visible of colloidal conjugates on specific areas of a solid porous support.

6. METHOD. MEASURING PROTOCOL

The samples were processed following up the instructions for use related to each commercial kit.

- CerTest *Clostridium difficile* antigen GDH (CerTest Biotec S.L, Spain).
- Viasure *Clostridium difficile* Real Time Detection Kit (CerTest Biotec S.L, Spain)

7. EQUIPMENTS

For the evaluation of **CerTest *Clostridium difficile antigen GDH*** for *Clostridium difficile* antigen GDH detection which are qualitative tests it was not necessary the use of any equipment (visual interpretation), only follow the procedure of instructions for use.

The qPCR (Viasure *Clostridium difficile* Real Time Detection kit) measurements were run at an Aria Mx Real Time PCR instrument, Model G8830-64001 with SN: MY165005276 (from Ag Plant Technologies/Agilent).

8. CALCULATIONS

The analysis has been performed by using Meta-DiSc 1.4, a freeware software to perform Meta-analysis of studies of evaluations of Diagnostic and Screening tests. The program has been developed by the Unit of Clinical Biostatistics team of the Ramón y Cajal Hospital in Madrid (Spain).

Meta-analysis is a two-stage process. In the first stage, a summary statistic is calculated for each study. Unlike controlled trials, in evaluation of diagnostic tests, each

study is summarized by a pair of statistics that measures the test's accuracy. The pair is usually either sensitivity and specificity or positive and negative likelihood ratios. In the second stage, the overall test accuracy indexes are calculated as the weighted average of these summary statistics.

For more information: "Zamora J, Abraira V, Muriel A, Khan KS, Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. BMC Medical Research Methodology 2006, 6:31".

9. METHOD AND COMMERCIAL KIT USED FOR COMPARISON

CerTest *Clostridium difficile* antigen GDH Card Test (CerTest). Cat. number: GD820001V.

Lot number used:

General lot	**GD-046**	**Expiry date**	**2019-04**
Strips lot	**GD-046**	**Expiry date**	**2019-04**
Diluent lot	**GD-046**	**Expiry date**	**2019-04**

Viasure *Clostridium difficile* Real Time Detection Kit (CerTest).

Cat. number: VS-CDS113L

Lot number used:

General lot	**CDS113L-005**	**Expiry date**	**2019-04**
Plate lot	**CDS1PL-005**	**Expiry date**	**2019-04**
Buffer lot	**RB02-011**	**Expiry date**	**2021-04**
Negative CTRL lot	**NC1-009**	**Expiry date**	**2021-04**
Positive CTRL lot	**CDS1C-002**	**Expiry date**	**2020-05**
Water lot	**H2O-009**	**Expiry date**	**2021-04**

Samples were tested following the kit instructions for use:

Product Name	IFU draft version
CerTest *Clostridium difficile* antigen GDH test, CerTest	IU-GD8V rev 04
Viasure *Clostridium difficile* Real Time Detection Ki, CerTest	VS-CDS12enes1115

All the products used are CE marked.

The evaluation was made following internal protocol: **CerTest *Clostridium difficile* antigen GDH evaluation protocol 020417.**

10.RESULTS AND DISCUSSION

Raw Data. Results Evaluation. Date: 17th April to 2nd June 2017 (*Clostridium difficile* antigen GDH)

Product name: CerTest *Clostridium difficile* antigen GDH,

Lot number: GD-046, expiry date: 2019-04.

This product is a card format, cat. reference: GD820001V and lot number GD-046.

The samples have been evaluated by ICtest and qPCR technique. qPCR has been considered as a Gold Standard.

126 frozen stool samples have been included in the study; 51 samples were positive ones; meanwhile 75 were negative.

The next table shows the results for the LF test as well as the results for qPCR (considered as a gold standard). Results interpretation has been done following the instructions from the providers.

No	Sample	GDH	
		qPCR Lot number: CDS113-L005	ICTest Lot number: GD-046
		Results: Cq (if sample is positive)	Results
1	12937	20.81	+
2	12497	20.82	+
3	12094	21.07	+
4	16452	22.40	+
5	12428	22.51	+
6	12675	23.92	+
7	6438	23.97	+
8	8930	24.29	+
9	11108	24.81	+
10	4988	26.10	+
11	9423	27.42	+
12	7896	25.79	+
13	11107	26.10	+
14	15931	22.00	+
15	6758	20.66	+
16	15931	22.00	+
17	12431	22.94	+
18	12499	27.28	+
19	7603	26.88	+
20	13029	24.74	+
21	15702	22.27	+
22	13876	22.47	+
23	13277	29.97	+
24	13873	29.97	+
25	9285	27.13	+
26	9285	27.13	+
27	13874	13.86	+
28	7751	20.51	+
29	11741	28.96	+
30	10493	23.56	+
31	10875	28.50	+
32	5556	26.98	+
33	9163	24.45	+
34	7756	24.97	+
35	9217	29.23	+
36	7751	20.51	+
37	6457	20.56	+
38	14703	22.30	+

No	Sample	GDH	
		qPCR Lot number: CDS113-L005	ICTest Lot number: GD-046
		Results: Cq (if sample is positive)	Results
39	10918	22.37	+
40	8615	26.37	+
41	16312	26.40	+
42	16364	26.50	+
43	15651	33.40	+
44	14184	24.56	+
45	14585	18.64	+
46	14701	19.00	+
47	9284	31.92	+
48	5307	39.09	+
49	7646	32.64	+
50	5587	31.65	+
51	12500	35.43	+
52	4623	-	-
53	5557	-	-
54	4442	-	-
55	4619	-	-
56	6771	-	-
57	14187	-	-
58	14462	-	-
59	14568	-	-
60	14569	-	-
61	14571	-	-
62	14573	-	-
63	14574	-	-
64	14576	-	-
65	14577	-	-
66	14578	-	-
67	14580	-	-
68	14582	-	-
69	14591	-	-
70	14595	-	-
71	14596	-	-
72	14614	-	-
73	14676	-	-
74	14687	-	-
75	14692	-	-
76	15164	-	-

No	Sample	GDH	
		qPCR Lot number: CDS113-L005	ICTest Lot number: GD-046
		Results: Cq (if sample is positive)	Results
77	15620	-	-
78	15661	-	-
79	15677	-	-
80	15684	-	-
81	15706	-	-
82	16302	-	-
83	16351	-	-
84	16353	-	-
85	16363	-	-
86	16369	-	-
87	16373	-	-
88	16411	-	-
89	16412	-	-
90	16413	-	-
91	16414	-	-
92	16416	-	-
93	16417	-	-
94	16418	-	-
95	16419	-	-
96	16420	-	-
97	16421	-	-
98	16422	-	-
99	16423	-	-
100	16424	-	-
101	16426	-	-
102	16427	-	-
103	16428	-	-
104	16429	-	-
105	16535	-	-
106	16536	-	-
107	16537	-	-
108	16538	-	-
109	16539	-	-
110	16540	-	-
111	16541	-	-
112	16542	-	-
113	16543	-	-
114	16545	-	-

No	Sample	GDH	
		qPCR Lot number: CDS113-L005	ICTest Lot number: GD-046
		Results: Cq (if sample is positive)	Results
115	16547	-	-
116	16548	-	-
117	16549	-	-
118	16552	-	-
119	16553	-	-
120	16554	-	-
121	16555	-	-
122	16556	-	-
123	16557	-	-
124	16558	-	-
125	16559	-	-
126	16686	-	-

Note: Results interpretation has been done at 10 minutes for Certest test (ICtest), in all cases following the test instructions from the provider.

From the previous raw data it can be determined that:

- 51 Samples (from sample No1 (12937) to sample No 51 (12500) at the table) were positive for Lateral flow test without discrepancy among them or with qPCR technique. They are considered all true *Clostridium difficile* antigen GDH
- The rest of the samples (from sample No 52, sample 4623 to sample No 126 (16686)) were negative for LF test and qPCR, showing no discrepancies and considered as negatives for GDH antigen.

11.EVALUATION SUMMARY

Viasure *Clostridium difficile* Real Time Detection Kit, CerTest has been considered as Gold Standard for the determination of the analytical sensitivity and specificity. Thus, analytical sensitivity and specificity refers to that test.

For statistical analysis of the results, the free-software package Meta DiSc ver 1.4 has been used (see paragraph 8). The Confidence Intervals calculated for sensitivity, specificity, positive and negative prediction values (PPV and NPV) were at 95% confidence.

Results:

CerTest *Clostridium difficile antigen GDH* test vs Viasure *Clostridium difficile* Real Time Detection Kit

		qPCR Viasure *Clostridium difficile* Real Time Detection Kit		
IC test: CerTest *Clostridium difficile* antigen GDH		+	-	Total
	+	51	0	51
	-	0	75	75
	Total	51	75	126

CerTest *Clostridium difficile* vs qPCR Viasure *Clostridium difficile* Real Time Detection kit		
	Mean Value	**95% CI (confidence interval)**
Sensitivity	100.0%	93.0 – 100.0%
Specificity	100.0%	95.2 – 100.0%
PPV	100.0%	93.0 - 100.0%
NPV	100.0%	95.2 –100.0%

Kappa value: 0.99 Excellent concordance value

12.CONCLUSION

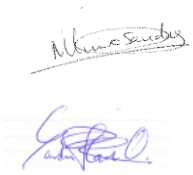
Values for **CerTest *Clostridium difficile* antigen GDH** test evaluation are good enough, as concluded after their comparison vs qPCR **Viasure *Clostridium difficile* Real Time Detection Kit.**

System	Sensitivity and CI	Specificity and CI	PVP and CI	NPV and CI
Certest *Clostridium difficile* antigen GDH vs Viasure *Clostridium difficile* Real Time Detection Kit.	100.0% 93.0 - 100.0%	100.0% 95.2 - 100.0%	100.0% 93.0 - 100.0%	100.0% 95.2 - 100.0%

Table: summary of results.

The obtained results of sensitivity and specificity conform to the expected ones.

The Certest *Clostridium difficile* antigen GDH test performance shows high sensitivity and specificity to detect the presence of *Clostridium difficile* antigen GDH *in stool samples.*

Name:	Made by:	Approved by:
	Marisa Sanchez & Sandra Garcia	Manuel Villacampa
Signed: Date:	 2nd June 2017	 2nd June 2017
Position:	Laboratory technicians	Immunochromatographic Manager