L-cysteine transporter-PCR to detect hydrogen sulfide-producing *Campylobacter fetus*

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L-Cysteine Transporter-PCR to Detect Hydrogen Sulfide-Producing *Campylobacter fetus*

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Abstract

Phenotypic differences between *Campylobacter fetus fetus* and *Campylobacter fetus venerealis* subspecies allow the differential diagnosis of bovine genital campylobacteriosis. The hydrogen sulfide production, for example, is a trait exclusive to *Campylobacter fetus fetus* and *Campylobacter fetus venerealis* biovar intermedius. This gas that can be biochemically tested can be produced from L-cysteine. Herein we report a novel multiplex-PCR to differentiate *Campylobacter fetus* based on the evaluation of a deletion of an ATP-binding cassette-type L-cysteine transporter that could be involved in hydrogen sulfide production, as previously described. A wet lab approach combined with an *in silico* whole genome data analysis showed complete agreement between this L-cysteine transporter-PCR and the hydrogen sulfide production biochemical test. This multiplex-PCR may complement the tests currently employed for the differential diagnosis of *Campylobacter fetus*.

Introduction

*Campylobacter fetus* (*C. fetus*) is best known as a major veterinary pathogen that has a detrimental effect on reproductive efficiency of herds. However, in humans, this bacterium can also cause intestinal illness and, occasionally, severe systemic infections and thus the products from cattle and sheep are suspected as sources of transmission (Wagenaar et al., 2014). The classification of *C. fetus* subspecies relies on clinical features, host specificity, and phenotypic traits. Despite technical limitations and variable success, hydrogen sulfide (*H₂S*) production as well as tolerance to glycine and NaCl, selenite reduction and resistance to antibiotics are the
available biochemical tests currently employed as differential diagnosis of *C. fetus* (OIE, 2018; Schulze et al., 2006).

Members of *C. fetus* have different tropism, as evidenced in veterinary practice and in the diagnosis. The subspecies *C. fetus venerealis* (Cfv) is restricted to the bovine reproductive tract, and is associated to the venereal disease bovine genital campylobacteriosis (BGC), whereas *C. fetus fetus* (Cff) is mainly intestinal and is usually related to sporadic abortion. To date, the bovine products are subjected to strict regulations by the World Organisation for Animal Health (OIE) and must be tested for the presence of *C. fetus* subsp. *venerealis* before international trading (OIE, 2019). Therefore, its differentiation at the subspecies level is critical. The isolation of the bacteria can confirm BGC and subsequently biochemical tests can determine the particular different isolates. Among the biochemical tests, glycine resistance and hydrogen sulfide (H$_2$S) production are two of the best biochemical performing tests. For example, Cff strains show 1% glycine resistance and produce H$_2$S in L-cysteine (L-Cys) enriched media. By contrast, Cfv strains fail to grow in 1% glycine-containing media and to produce H$_2$S (Véron and Chatelain, 1973). Hence, these traits allow their discrimination. A glycine-tolerant variant of Cfv (*C. fetus venerealis* biovar intermedius, Cfvi) are frequently isolated in some countries such as USA, UK, South Africa and Argentina, which complicates their accurate identification (Schmidt et al., 2010; van Bergen et al., 2005; Iraola et al., 2013). A third-host associated subspecies, *C. fetus* subsp. *testudinum*, completes the list of subspecies of *C. fetus*. This subspecies has been isolated from reptiles and humans (Fitzgerald et al., 2014) and therefore would not be relevant for animal production.

In a previous wide genome association study, van der Graaf-van Bloois et al. described a recent diversification of mammalian *C. fetus* and implicated a genetic factor associated to H$_2$S
production (van der Graaf–van Bloois et al., 2016). They described a deletion in an ATP-binding cassette-type L-Cys transporter in Cfv strains. The operon structure of this L-Cys transporter has five coding sequences and three of them code for different molecular components of the transporter: the ATP-binding protein, the permease, and the substrate-binding protein (locus tags CFF8240_RS03845, CFF8240_RS03850 and CFF8240_RS03855 in C. fetus 82-40 genome, respectively). This L-Cys importer could be part of the Class 3 ABC-transporters (Licht and Schneider, 2011) and in Cfv the permease and the extracellular binding domain coding genes are deleted. This deletion may impair the transporter assembly, affecting the up-take of L-Cys. This therefore could explain the impaired production of H2S from this amino acid in Cfv strains. On these bases, we aimed to develop a simple molecular technique for detecting the L-Cys transporter-deletion polymorphism with the main purpose of identifying H2S-producing C. fetus strains.

**Materials & Methods**

**C. fetus Isolates and Bacterial Culture.** All the C. fetus isolates (n=36) were obtained from bovine clinical samples at the Bacteriology Unit (EEA-INTA Balcarce, Argentina). Thirty of these clinical isolates were randomly-selected for this study. In addition, the strains Cfv 97/608, Cfv 98/25 and Cfvi 99/541 were also selected because of the availability of their whole genome sequences (van der Graaf et al., 2014; Iraola et al., 2013) and three additional isolates were selected to perform whole genome sequencing (see below).

All the C. fetus isolates were grown on 7% blood-Skirrow selective agar plates (Oxoid) with 1.25 IU/ml polymyxin B sulphate, 5 μg/ml trimethoprim, 10 μg/ml vancomycin and 50 μg/ml cycloheximide (Sigma). The plates were incubated under microaerophilic conditions (5% O2,
10% CO₂ and 85% N₂) for 72h at 37°C. Campylobacter hyointestinalis NCTC11562 and the field isolate Campylobacter sputorum 08/209 were grown under the same conditions. Campylobacter coli NCTC11353 and Campylobacter jejuni NCTC11392 were cultured on Blood-Columbia agar plates (Oxoid) under microaerophilic condition for 24h at 42°C.

Biochemical Tests. The classification of the subspecies was performed following standard protocols (OIE, 2018): sodium selenite reduction, 3.5% sodium chloride resistance, 1% glycine tolerance and H₂S production in 0.02% L-cysteine enriched medium. We also tested 1.3%, 1.5% and 1.9% glycine tolerance. The isolates were identified as Cff if they reduced sodium selenite, produced H₂S and showed sodium chloride tolerance and at least 1% glycine resistance.

DNA Isolation. A rapid protocol (freeze-thaw cycles) was applied to obtain the DNA template as follows. A loopful of each culture was collected and resuspended in 250 µl of sterile deionized water. Two cycles of freeze and boiling (95°C/-80°C) were performed and the cellular debris were discarded after a centrifugation step. Two µl of the supernatant was used as PCR-template. High quality genomic DNA was obtained using mini spin columns (NucleoSpin Tissue, Macherey-Nagel GmbH & Co.). DNA quality was tested using the Qubit 4 fluorometer (Invitrogen, Thermo Scientific) and further used for sequencing purpose.

L-Cys Transporter-PCR. One forward and two reverse primers (For 5’gtccatttacattacagtaacagtgg 3’, Rev1 5’gatattaggctaagaggaatggtattg 3’ and Rev2 5’ctcccgtatcatcaagctaatate 3’) were designed for a multiplex-PCR format using open source Unipro UGENE 1.31 (Okonechnikov et al., 2012) (Fig. 1A). The amplification mix consisted of 1×GoTaq green Reaction buffer (1.5mM MgCl₂), 0.25 mM of each dNTP, 0.1 µM of each primer, and 1.25 U Taq polymerase (Promega Corp.), nuclease-free water to reach a final volume of 25 µl and Campylobacter DNA template.
The touch-down amplification program consisted of an initial step at 94°C for 3 min, 10 cycles at 94°C for 1 min, followed by annealing temperatures starting at 55°C for 1 min and decreasing 1°C per cycle from 55°C to 45°C. Then, an extension step was performed at 72°C for 1 min, followed by 30 cycles with an annealing at 51°C, and a final termination step at 72°C for 8 min. Under these conditions, the absence of the expected product of 1,390 bp makes the interpretation of the PCR results easy. A product of 714 bp is indicative of Cff and Cfvi strains (which have a complete version of the operon and are H₂S-producing strains), whereas a 310 bp product refers to Cfv strains (which contain a partly deleted operon and are non- H₂S-producing strains). All the products were resolved in 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The PCR-products were submitted to the UGB unit-INTA to confirm their identity through Sanger sequencing.

**In silico-PCR: Whole Genome Sequencing and Genomic Data Analysis.** We selected three isolates from bovine abortions (Cff 13/344, Cff 08/421 and Cfvi 06/341) of the most productive agricultural areas of Argentina. Paired-end Nextera XT libraries were constructed and sequenced in a MiSeq sequencer (2x250 pb, Illumina). A quality trimming step was applied to raw reads using Trimmomatic (Bolger et al., 2014). De novo assembly was done using SPAdes v3.11.1 (Bankevich et al., 2012). Contigs were oriented using Mauve (Darling et al., 2004; Rissman et al., 2009) and the genome of *Campylobacter fetus venerealis* 97-608 as a reference (NZ_CP008810.1). The genomes were annotated using PROKKA (Seemann, 2014) and RASTtk (Brettin et al., 2015). The assembly summary statistics is shown in Table S1.

In total, whole-genome sequence data of 214 *C. fetus* strains (Cff, n=152; CfV, n=42; Cfvi, n=19 and one strain not identified at the subspecies level, Cf=1) from 19 countries and different hosts (bovine, n=117; human, n=78; ovine, n=15; monkey, n=1 and unknown, n=3) were screened to
search for the target sequences of the primers designed for the L-Cys transporter-PCR protocol. These data included the three genomes obtained in this study (Cff 13/344, Cff 08/421 and Cfvi 06/341) and thirty-seven publicly available genomes from GenBank. Additionally, reads from C. fetus strains (n=174) deposited in ENA database (http://www.ebi.ac.uk/ena/) were also assembled, as mentioned above, and subsequently analysed as follows. The Primer map software (http://www.bioinformatics.org/sms2/primer_map.html) was used for global searching of For, Rev1 and Rev2 primer sequences. Primer Map output is a textual map showing the annealing positions of PCR primers. Afterwards, several conditions were evaluated, including annealing of both primers of each pair and their orientation. The position of each target annealing site was employed to estimate the amplicon size. The program, by default, does not allow mismatches. Cases where the annealing was confirmed for a single primer were classified as not detected or unknown.

Statistics. The agreement between the H₂S production biochemical test and the L-Cys transporter-PCR was tested with Cohen’s Kappa statistic.

Results

L-Cys transporter-PCR: Wet-Lab Assay. The multiplexed PCR-based approach herein designed produced a differential band pattern between the C. fetus isolates with distinct H₂S-biochemical test results (Fig. 1B). This protocol was named L-Cys transporter-PCR. We tested 36 biochemically typed isolates with this L-Cys transporter-PCR, followed by electrophoresis of the products in agarose gel to reveal the size of the amplicons. A single amplification product was obtained in all the tested strains. The retrieved band from Cff and Cfvi biovar intermedius (Cfvi) strains was of 714 bp. This result coincided with a complete version of the L-Cys
transporter operon and this pattern was named “CFF/CFVI”. Amplifications from CfV strains generated a smaller product of 310 bp, equivalent to a partially deleted operon, and this profile was named “CFV” (Fig. 1B). This L-Cys transporter-PCR allowed a differential testing that avoided a negative result in presence of *C. fetus* DNA. Indeed, a negative result, sometimes could be indicative of both the absence of the specific target and the presence of inhibitors in the sample. As expected, no product was obtained from DNA of *Campylobacter* spp. other than *C. fetus* (*C. hyointestinalis*, *C. coli*, *C. jejuni* and *C. sputorum*) (Table 1). This result confirmed the specificity of this L-Cys transporter-PCR test for *C. fetus*.

The results from the L-Cys transporter-PCR analysis displayed a perfect correlation with the H$_2$S production test (kappa=1). The analysis of concordance between tests is shown in Table S2.

We also addressed an *in silico* analysis of genomic sequences from mammalian *C. fetus* to further support this conclusion.

**L-Cys Transporter-PCR: in silico Screening.** To study the performance of the L-Cys transporter-PCR in a more diverse panel of strains, we applied an *in-silico* PCR-strategy by performing searches of the primer targeting sequences in whole genomes of 214 *C. fetus* strains (three of which were obtained in this study by Next-Generation Sequencing technology). For this purpose, we employed the online Primer map application. The same products as the obtained by the wet lab-PCR were considered among all the predicted PCR products and the same patterns were determined according to the product size. This approach confirmed the primer annealing sites, and consequently, also allowed us to define the type of L-Cys transporter operon in 213 out of 214 *C. fetus* strains (Table 2). The target annealing sites were highly conserved because of the lack of nucleotide mismatches in these strains. The *in silico*-PCR was able to predict the annealing sites for For and Rev2 primers in the genome of CfV Azul-94 but the target sites were
located into different contigs. The product size was difficult to estimate and consequently this
strain had inconclusive results (Table 2).

The hydrogen sulfide production data were available for 43/214 of the studied strains, three from
this study and forty from publicly available results (van Bergen et al., 2014; van der Graaf-van
Bloois et al., 2014, 2016; Willoughby et al., 2005). However, two of the evaluated strains have
shown discrepant results according to the literature and were excluded from this analysis.

Interestingly, all of the H$_2$S-producing strains displayed a CFF/CFVI pattern, whereas the non-
H$_2$S-producing strains, with unequivocally results according to the biochemical test, presented a
CFV pattern (k=1) (Table 2). The analysis of concordance between in silico-PCR and H$_2$S
production is shown in Table S3.

Despite this concordance with the H$_2$S-production test, 14 out of 43 strains that were identified
as Cfv in the database did not match with the criteria of the deleted L-Cys transporter for this
subspecies. Instead, these strains displayed a CFF/CFVI pattern (Table 2). This situation is also
reflected by the overall analysis where the in silico study was able to assign the expected result
in 92 % (197/213) of the strains (one strain with inconclusive subspecies identification was
excluded from the analysis). This partial discrepancy could be attributed to the different methods
employed to determine subspecies and this information is not available for most of the strains
used in this analysis.

As a proof of concept, we assessed six local field isolates (Cff 08-421, Cff 13-344, Cfv 97-608,
Cfv 98-25, Cfvi INTA 99/541 and Cfvi 06-341) through the wet-lab and in silico-PCR
approaches. The strains Cfv 97-608, Cfv 98-25 and Cfvi INTA 99/541 were selected from the
Campylobacter fetus collection because of their genomic sequence availability (van der Graaf-
van Bloois et al., 2016; Iraola et al., 2013). The L-Cys-transporter-PCR results perfectly matched the in silico-PCR predictions (Tables 1 and 2).

Altogether, this study showed a strong concordance between the results of the L-Cys transporter-PCR and the H$_2$S-production test for C. fetus analysis. Furthermore, it highlights the lack of consensus in the classification of these bacteria between the different laboratories around the world.

Discussion

To date, phenotypic tests are among the most valuable methods to identify and differentiate microorganisms. However, these tests are usually time-consuming, because they are growth-rate dependent, and the whole process depends on the objectivity and skills of the operator. Furthermore, a proper standardization, which is essential to obtain reliable and reproducible results, is often absent. Despite all this, the replacement of these phenotypic tests by molecular techniques is not always an alternative to date. The antimicrobial resistance constitutes a good example of complementary testing, and this particular phenotypic trait can be tested by bacteriological methods and at molecular level in several pathogens (Fluit et al., 2001).

Over the last years, researchers have proposed many genotypic tests to facilitate C. fetus differentiation. For example, different studies have employed molecular techniques such as PCR based on different target genes to differentiate Cf$_v$ from Cf$_f$ (Hum et al., 1997; van Bergen et al., 2005; Abril et al., 2007). However, to date there is no clear consensus on the best method to assess C. fetus subspecies. The main problems rise from the limited number of tested strains, the failure to identify Cf$_v_i$ strains and the low concordance with other techniques such as AFLP and, mainly, biochemical tests (Willoughby et al., 2005; Schultze et al., 2006; Schmidt et al., 2010).
A genome-wide association study has proposed the association between candidate gene loci coding for the L-Cys transporter and the H$_2$S production, which together to glycine resistance is one of the phenotypic traits available for assessing *C. fetus* subspecies to date. According to this, H$_2$S-producing *C. fetus* strains, commonly classified as Cff and Cfvi, have a complete L-Cys transporter operon, whereas the non-producing H$_2$S *C. fetus* strains, classically classified as Cfv, have a deleted L-Cys transporter. It is important to mention that *C. fetus* subsp. *testudinum* (Cft), the last subspecies proposed of *C. fetus*, has a complete version of the operon. This is the case of the strain Cft 03/427 (whose genome is the representative of the species) which has been concordantly described as an H$_2$S-producing strain elsewhere (van der Graaf-van Bloois et al., 2016). To date, this subspecies has not been described in cattle, and for this reason it was excluded of this study.

In this work, we have designed a multiplex-PCR protocol to provide a molecular tool to contribute to *C. fetus* characterisation and differentiation. This L-Cys transporter-PCR showed an excellent correlation with the H$_2$S production test according to both wet lab and *in silico* approaches. As other molecular techniques, this PCR failed to differentiate Cff from Cfvi strains. This will limit its use in countries where this biovar is prevalent. However, until more discriminative techniques are developed, its usefulness could be further enhanced by combining this technique with other complementary test, such as the glycine resistance assay.

In addition to practical implications of this tool in the laboratory, this study also contributes to the existing debate around *C. fetus* subspecies classification.

In this study, we tested *C. fetus* strains isolated and typed at the Bacteriology Unit of INTA-Balcarce (Argentina), which has a long history in culturing this bacterium and in performing its biochemical based classification. In this way, the wet-lab approach showed a perfect correlation
not only with the H$_2$S production test, but also with the *C. fetus* subspecies. Indeed, a CFF-CFVI pattern, which is indicative of L-Cys complete transporter, was associated with H$_2$S-producing strains typically classified as Cff or Cfvi. By contrast, a CFV pattern, which is indicative of a deleted transporter, was exclusively associated with H$_2$S-non-producing strains, which are typically classified as Cfv.

On the other hand, when we performed the *in silico* study, we analysed genomic data from strains classified elsewhere by both molecular based approaches and/or biochemical tests and, as mentioned above, both techniques frequently displayed discordant results. In this way, we have obtained a perfect correlation with the H$_2$S production test, but not with the reported subspecies of the strains.

This discordancy is well reflected by the strain 98-25. Researchers from the Bacteriology Unit of INTA-Balcarce isolated this strain in 1998 from aborted foetus, and originally typed it as Cfv because of its glycine sensitivity and its inability to produce H$_2$S. This strain was included in this study and the PCR-L-Cys result was concordant with the phenotype of this strain. Later studies have also tested this strain and successfully sequenced its genome. Indeed, van Bergen et al., (2005) typed it as glycine sensitive and H$_2$S positive (typical traits of Cfvi strains). Later, van der Graaf et al., (2014) reported it as non-H$_2$S-producing strain. However, in this latter work, it has been called Cfvi 98-25 regardless the biochemical traits reported. In our study, the *in silico* sequence data analysis revealed the polymorphism of the L-Cys transporter (CFV pattern) typical of the non- H$_2$S- producing strains.

Therefore, the same strain could display different biochemical traits when assayed in different labs -or time- and this is the classical bottle-neck of phenotypic tests.
We initially had other discrepancies with some isolates. Remarkably, 14 out of 43 CfV genomic sequences tested in silico showed a complete version of the L-Cys transporter (CFF/CFVI pattern). Hence, at first glance, the hypothesis that all CfV isolates do have a deleted L-Cys transporter appeared as not valid, according to the in silico analysis. However, when we searched the biochemical tests reported for some of these strains, we concluded that the in silico results presented here were concordant with the H$_2$S production test. This discrepancy with the subspecies assigned could be due to the classification method of the strains that is frequently based on molecular techniques regardless the biochemical test results and moreover; the chosen method is not always specified (Iraola et al., 2017). Altogether, the in silico analysis also supports the hypothesis that states the occurrence of a deletion in the transporter operon in non-H$_2$S producing strains, which are classified as CfV according to biochemical methods.

As was mentioned earlier, it is important to highlight that the strains from databases are not typed by the same methodology and this fact is not always taken into account. Consequently, this could be problematic as our study showed. The most widespread molecular-based method is the multiplex-PCR described by Hum et al. (1997). This PCR targets the parA gene to identify CfV strains. This transfer-associated gene is harboured in a pathogenicity island which encodes a Type 4 Secretion System (T4SS). Although the presence of a T4SS has been previously associated to CfV strains (Gorkiewicz et al., 2010), it has also been demonstrated later that some Cff strains can harbour the T4SS and their related genes (van der Graaf-van Bloois et al., 2016). Furthermore, distinct phylogenetic analyses of C. fetus suggest that the current classification in subspecies must be redefined. A phylogenomic study based on the core genome have shown that the strains are divided in two clusters. Whereas all the CfV and CfVI strains were grouped in one genome cluster, the Cff strains were equally distributed in both clusters (van der Graaf-van
Bloois et al., 2014). Additionally, a phylogenetic reconstruction based on the divergence acquired by recombination have also shown that Cf v and C f f strains share the same clade, which differs clearly from the clade of C f t strains of reptile origin (Gilbert et al., 2018). This emphasizes a real need to go further toward current C. fetus classification and identification, which will have a significant impact on the diagnostic practice. As mentioned above, although this issue has been addressed in the literature and genomic studies have broadened and strengthened our knowledge of this bacterium (van der Graaf-van Bloois et al., 2014; van der Graaf-van Bloois et al., 2016; Iraola et al., 2017), a concerted action toward C. fetus subspecies classification and differentiation has been neglected. There are no molecular markers associated to tropism or virulence of Cf v that could help with a differential diagnosis of the bovine genital campylobacteriosis (Gilbert et al., 2018).

Consequently, in light of these evidences, more research is essential to determine, as a first step, whether the differential diagnosis should be promoted and, if so, to improve or replace definitively the tests currently available. Another point to consider is that, veterinary diagnostic laboratories from developing countries are often refractory to replace those methods that have proven be useful, even if they are not the most suitable ones. Because of that, the adoption of genetic or even genomic-based methods has been delayed. One possible reason is the cost related to each method. The second main reason is the time lapse it takes for scientific knowledge to reach end users. Interdisciplinary research combining genomics, biochemistry, epidemiology and the provision of updated information and training to end users could shed light on this matter in the near future.

Conclusions
Biochemical tests including tolerance to glycine and H$_2$S production are currently recommended by the OIE (2018) for *C. fetus* subspecies differentiation and are still employed in laboratories around the world. Thus, a molecular tool linked to a phenotypic trait is a valuable tool that could be more accurate and less time consuming than the available phenotypic tests. Mutagenesis and functional studies are essential to associate definitely this putative L-Cys transporter with the H$_2$S production. Meanwhile, this study shows that this transporter constitutes a good marker that is useful for detecting H$_2$S-producing *C. fetus*. Future actions will be addressed to test the L-Cys-PCR in clinical samples to propose it not only as a typing method, but as a detection technique and, as a second phase of validation, to transfer this technology to other labs to test the reproducibility of the results.

Finally, this work provides a molecular tool linked to H$_2$S production in *C. fetus* and supports the findings of the pioneering study of van deer Graaf-van Bloois et al. (2016).

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Figure 1

Differential L-Cys Transporter-PCR

(A) Schematic representation of the organization of the genes encoding the L-Cys transporter in *C. fetus*. Primer targeting regions and expected PCR-products are shown. The grey arrow represents the permease protein YckJ coding gene (locus tag CFF8240_RS03850 in Cff 82-40 genome) which is deleted in Cfvi strains. The light grey arrow represents the extracellular-binding protein YckK coding gene (locus tag CFF8240_RS03855), which is partially deleted in Cfvi. ATP-binding protein coding gene (locus tag CFF8240_RS03845) which is another component of the transporter is conserved in both subspecies and is represented by black arrow. (B) Representative agarose gel electrophoresis. Lane 1, negative control (water); lane 2, Cff 08/421; lane 3, Cff 96/136; lane 4, Cfvi 06/341; lane 5, Cfvi 97/608; lane 6, Cfvi 03/596 and lane 7, Cfvi 95/258. Under the set conditions, the product of 1,390 bp is absent. M: molecular weight marker, 1kb ladder (Promega).
A

Cff

Rev2

Rev1

Fwd

1,390 bp

714 bp

Cfv

Rev2

Fwd

310 bp

B

M 1 2 3 4 5 6 7

714 bp (Fwd-Rev1)

310 bp (Fwd-Rev2)
Table 1 (on next page)

L-Cys Transporter-PCR: Analysis of Argentinian *C. fetus* Isolates and *Campylobacter* spp. Strains.

“CFF/CFVI pattern” means that all the components of the L-Cys transporter are present and therefore, a product of 714 bp is obtained. “CFV pattern” means that the L-Cys transporter is deleted and a product of 310 bp is obtained. “-” means that the amplification product was absent. BA: Buenos Aires province, LP: La Pampa province, SF: Santa Fe province. ND: Not determined
| Strain  | Origin         | Biochemical test | Phenotypic ID | L-Cys Transporter-PCR Pattern |
|---------|----------------|------------------|---------------|-----------------------------|
|         |                | 1% glycine resistance |                |                             |
|         |                | H₂S Production |               |                             |
|cff 96-136 | Bahía Blanca, BA | +                 | +             | Cff                         |
|cff 08-421 | Gral. López, SF  | +                 | +             | Cff                         |
|cff 14-284 | Pila, BA        | +                 | +             | Cff                         |
|cff 04-240 | Olavarria, BA   | +                 | +             | Cff                         |
|cff 13-344 | Balcarce, BA    | +                 | +             | Cff                         |
|cff 11-572 | Balcarce, BA    | +                 | +             | Cff                         |
|cff 89-222 | Balcarce, BA    | +                 | +             | Cff                         |
|cff 90-189 | Balcarce, BA    | +                 | +             | Cff                         |
|cff CI N3  | Balcarce, BA    | +                 | +             | Cff                         |
|cff 01-165 | Santa Rosa, LP  | +                 | +             | Cff                         |
|cff 01-64  | Balcarce, BA    | +                 | +             | Cff                         |
|cff 05-622 | Cnel. Dorrego, BA| +                 | +             | Cff                         |
|cff 11-262 | Balcarce, BA    | +                 | +             | Cff                         |
|cff 11-295 | Saladillo, BA   | +                 | +             | Cff                         |
|cff 11-360 | Necochea, BA    | +                 | +             | Cff                         |
|cff 11-685 | Balcarce, BA    | +                 | +             | Cff                         |
|cff 11-408 | Necochea, BA    | +                 | +             | Cff                         |
|cff btu5   | BA              | +                 | +             | Cff                         |
|cff btu6   | BA              | +                 | +             | Cff                         |
|cff btu7   | BA              | +                 | +             | Cff                         |
|cff 18-09  | BA              | +                 | +             | Cff                         |
|cff 18-100 | BA              | +                 | +             | Cff                         |
|cff 97-608 | Hucal, LP       | -                 | -             | Cfv                         |
|cff 95-258 | Mar Chiquita, BA| -                 | -             | Cfv                         |
|cff 08-382 | Gral. Belgrano, BA| -               | -             | Cfv                         |
|cff 05-355 | Balcarce, BA    | -                 | -             | Cfv                         |
|cff 98-25  | Gral. Pueyrredón, BA| -             | -             | Cfv                         |
|cff 19-01  | BA              | -                 | -             | Cfv                         |
|cffi 06-341| Pehuajó, BA     | -                 | +             | Cff                         |
|cffi 03-596| Pehuajó, BA     | -                 | +             | Cff                         |
|cffi 02-146| BA              | -                 | +             | Cff                         |
|cffi 98-472| Azul, BA        | -                 | +             | Cff                         |
|cffi 99-541| Balcarce, BA    | -                 | +             | Cff                         |
|cffi 07-379| Mar Chiquita, BA| -                 | +             | Cff                         |
|cffi 00-305| BA              | -                 | +             | Cff                         |
|cffi 03-596| Pehuajó, BA     | -                 | +             | Cff                         |
|c. sputorum 08-209 | Balcarce, BA | ND    | ND            | ND                          |
|c. coli NCTC11353 | National Collection of Type Cultures, England | ND    | ND            | ND                          |
|c. hyointestinalis NCTC11562 | National Collection of Type Cultures, England | ND    | ND            | ND                          |
|c. jejuni NCTC11392 | National Collection of Type Cultures, England | ND    | ND            | ND                          |
Table 2 (on next page)

**In silico-PCR: Analysis of Whole-Genome Sequence Data.**

“CFF/CFVI pattern” means that a complete L-Cys transporter is present. Hybridization of the primer pair For-Rev1-template should occur and a product of 714 bp is predicted. “CFV pattern” means that the L-Cys transporter is partially deleted. Hybridization of the primer pair For-Rev2-template should occur and a product of 310 bp is predicted. Country code: US, United States; AR, Argentina; UK, United Kingdom; CZ, Czech Republic; FR, France; AU, Australia; CA, Canada; SA, South Africa; NL, The Netherlands; UY, Uruguay; BE, Belgium; IR, Ireland; IN, India; TR, Turkey; JP, Japan; TW, Taiwan; SP, Spain; GE, Germany; BR, Brazil. N.A: Not available
| ID | Strain | Host       | Source | Country | Accession number       | H$_2$S Production (Reference) | PCR L-Cys Transporter Pattern |
|----|--------|------------|--------|---------|------------------------|-------------------------------|-------------------------------|
| Cff 04/554 | Bovine | foetus     | AR     |         | CP008808-CP008809      | +                             | (van der Graaf-van Bloois et al., 2014) | CFF/CFVI |
| Cff 08/421 | Bovine | foetus     | AR     |         | SOOT000000000          | +                             | (This study)                  | CFF/CFVI |
| Cff 110800-21-2 | Bovine | bull     | NL     |         | LSZN000000000          | +                             | (van der Graaf-van Bloois et al., 2014) | CFF/CFVI |
| Cff 13/344 | Bovine | foetus     | AR     |         | SOYX000000000          | +                             | (This study)                  | CFF/CFVI |
| Cff 82/40  | Human  | blood     | US     |         | CP000487               | +                             | (van Bergen et al., 2005)     | CFF/CFVI |
| Cff Cff 98/v445 | Bovine | foetus     | UK     |         | LMBH000000000          | +                             | (van Bergen et al., 2005)     | CFF/CFVI |
| Cff ATCC 27374 | Ovine  | foetus (brain) | unk.  |         | MKEI000000000          | +                             | (van Bergen et al., 2005)     | CFF/CFVI |
| Cff BT 10/98 | Ovine  | unknown   | UK     |         | LRAL000000000          | +                             | (van Bergen et al., 2005)     | CFF/CFVI |
| Cff NCTC10842 | unknown | unknown | unk.  |         | LS483431               | +                             | (van Bergen et al., 2005)     | CFF/CFVI |
| Cff B0042 | Bovine | feces     | UK     |         | ERR419595              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0047 | Bovine | feces     | UK     |         | ERR419600              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0066 | Bovine | feces     | UK     |         | ERR419653              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0097 | Bovine | feces     | UK     |         | ERR419653              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0129 | Bovine | feces     | UK     |         | ERR419637              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0130 | Bovine | feces     | UK     |         | ERR419638              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0131 | Bovine | feces     | UK     |         | ERR419639              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0151 | Bovine | feces     | UK     |         | ERR419648              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0152 | Bovine | feces     | UK     |         | ERR419649              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0167 | Bovine | feces     | UK     |         | ERR460866              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff B0168 | Bovine | feces     | UK     |         | ERR460867              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff S0693A | Bovine | feces     | UK     |         | ERR419284              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cff S0478D | Bovine | feces     | UK     |         | ERR419653              | +                             | (van der Graaf-van Bloois et al., 2016) | CFF/CFVI |
| Cffv 01/165 | Bovine | mucus     | AR     |         | CP014568-CP014570      | +                             | (van Bergen et al., 2005)     | CFF/CFVI |
| Cfv 84/112 | Bovine | genital secretion | US  |         | HG004426-HG004427     | -                             | (van Bergen et al., 2005)     | CFV |
| Cfv  | 97/608   | Bovine  | placenta | AR | CP008810-CP008812 | (van Bergen et al., 2005) | CFV      |
|------|---------|---------|----------|----|-------------------|---------------------------|----------|
| Cfv  | ADRI 1362 | Bovine  | unknown  | AR | LREX000000000+    | (van der Graaf-van Bloois et al., 2014) | CFF/CFVI |
| Cfv  | B10     | Bovine  | unknown  | US | LRET000000000-    | (van der Graaf-van Bloois et al., 2014) | CFV      |
| Cfv  | CCUG 33872 | Bovine  | abortion | CZ | LREU000000000+/+   | (Willoughby et al., 2005; van der Graaf-van Bloois et al., 2014) | CFF/CFVI |
| Cfv  | CCUG 33900 | Bovine  | abortion | FR | LREV000000000-    | (van der Graaf-van Bloois et al., 2014) | CFV      |
| Cfv  | LMG 6570 | Bovine  | unknown  | BE | LREW000000000-    | (van Bergen et al., 2005) | CFV      |
| Cfv  | NCTC 10354 | Bovine  | mucus    | UK | CM001228          | (van Bergen et al., 2005) | CFV      |
| Cfv  | WBT 011/09 unknown | unknown | unknown  | UK | LMBI000000000+    | (van der Graaf-van Bloois et al., 2014) | CFF/CFVI |
| Cfv  | zaf3    | Bovine  | foetus    | SA | LREY000000000+    | (van der Graaf-van Bloois et al., 2014) | CFF/CFVI |
| Cfv  | zaf65   | Bovine  | unknown  | SA | LREV000000000+    | (van der Graaf-van Bloois et al., 2014) | CFF/CFVI |
| Cfv  | 02/298  | Bovine  | foetus    | AR | LRVK000000000+    | (van Bergen et al., 2005) | CFF/CFVI |
| Cfv  | 03/293  | Bovine  | foetus    | AR | LREV000000000+    | (van Bergen et al., 2005) | CFF/CFVI |
| Cfv  | 03/596  | Bovine  | foetus    | AR | LRAM000000000+    | (van Bergen et al., 2005) | CFF/CFVI |
| Cfv  | 06/341  | Bovine  | foetus    | AR | SOYW000000000+    | (This study) | CFF/CFVI |
| Cfv  | 92/203  | Bovine  | placenta  | AR | LRVLO000000000+   | (van Bergen et al., 2005) | CFF/CFVI |
| Cfv  | 97/532  | Bovine  | mucus     | AR | LRER000000000+    | (van Bergen et al., 2005) | CFF/CFVI |
| Cfv/Cfv | 98/25   | Bovine  | foetus    | AR | LRES000000000+/+/- | (van Bergen et al., 2005; van der Graaf-van Bloois et al., 2016; This study) | CFV      |
| Cfv  | 99/541  | Bovine  | prepuce   | AR | ASTK000000000+    | (van Bergen et al., 2005) | CFF/CFVI |
| Cff  | HC1-UY  | Human   | blood     | YU | JYCP000000000 n.a  | (van Bergen et al., 2005) | CFF/CFVI |
| Cff  | HC2     | Human   | cerebrospinal fluid | YU | QJTS000000000 n.a  | (van Bergen et al., 2005) | CFF/CFVI |
| Cff  | CIT01   | Human   | peripheral blood culture | IR | RBHV000000000 n.a  | (van Bergen et al., 2005) | CFF/CFVI |
| Cfv  | 642-21  | Bovine  | uterus    | AU | AJSG000000000 n.a  | (van Bergen et al., 2005) | CFF/CFVI |
| Cfv  | 66Y     | Bovine  | prepuce   | CA | JPQC000000000 n.a  | (van Bergen et al., 2005) | CFF/CFVI |
| Cfv  | Azul-94 | Bovine  | abortion  | AR | ACLG000000000 n.a  | (van Bergen et al., 2005) | CFV      |
| Cfv  | B6      | Bovine  | vagina    | AU | AJMC000000000 n.a  | (van Bergen et al., 2005) | CFV      |
| Cfv | TD   | Species       | Sample | CA  | Accession  | n.a. | CFV        |
|-----|------|---------------|--------|-----|------------|------|------------|
| Cf  | MM01 | Human         | sepsis | IN  | JRKX00000000 | n.a. | CFV/CFVI   |
| Cff | 99/801 | Bovine   | prepuce | AR  | ERS739235  | n.a. | CFV/CFVI   |
| Cff | 00/398 | Bovine   | foetus  | AR  | ERS739236  | n.a. | CFV/CFVI   |
| Cff | 00/564 | Bovine   | prepuce | AR  | ERS739237  | n.a. | CFV/CFVI   |
| Cff | 01/320 | Bovine   | foetus  | AR  | ERS739238  | n.a. | CFV/CFVI   |
| Cff | 01/210 | Bovine   | vaginal mucus | AR  | ERS739239  | n.a. | CFV/CFVI   |
| Cff | 04/875 | Bovine   | foetus  | AR  | ERS739242  | n.a. | CFV/CFVI   |
| Cff | 05/394 | Bovine   | foetus  | AR  | ERS739243  | n.a. | CFV/CFVI   |
| Cff | 05/434 | Bovine   | vaginal mucus | AR  | ERS739244  | n.a. | CFV/CFVI   |
| Cff | 06/340 | Bovine   | prepuce | AR  | ERS739245  | n.a. | CFV/CFVI   |
| Cff | 07/485 | Bovine   | vaginal mucus | AR  | ERS739248  | n.a. | CFV/CFVI   |
| Cff | 08/362 | Bovine   | foetus  | AR  | ERS739249  | n.a. | CFV/CFVI   |
| Cff | 10/247 | Bovine   | prepuce | AR  | ERS739250  | n.a. | CFV/CFVI   |
| Cff | 10/445 | Bovine   | prepuce | AR  | ERS739251  | n.a. | CFV/CFVI   |
| Cff | 11/360 | Bovine   | foetus  | AR  | ERS739252  | n.a. | CFV/CFVI   |
| Cff | 11/427 | Bovine   | vaginal mucus | AR  | ERS739253  | n.a. | CFV/CFVI   |
| Cff | 14/270 | Bovine   | foetus  | AR  | ERS739254  | n.a. | CFV/CFVI   |
| Cff | 15/301 | Bovine   | vaginal mucus | AR  | ERS739255  | n.a. | CFV/CFVI   |
| Cff | 02/146 | Bovine   | foetus  | AR  | ERS739240  | n.a. | CFV/CFVI   |
| Cff | 06/195 | Bovine   | foetus  | AR  | ERS739246  | n.a. | CFV/CFVI   |
| Cff | 07/379 | Bovine   | foetus  | AR  | ERS739247  | n.a. | CFV/CFVI   |
| Cff | 2006/367h | Human | cerebrospinal fluid | FR  | ERS672242  | n.a. | CFV/CFVI   |
| Cff | 2006/479h | Human | feces  | FR  | ERS672243  | n.a. | CFV/CFVI   |
| Cff | 2006/588h | Human | cerebrospinal fluid | FR  | ERS672244  | n.a. | CFV/CFVI   |
| Cff | 2006/621h | Human | blood  | FR  | ERS672245  | n.a. | CFV/CFVI   |
| Cff | 2006/649h | Human | feces  | FR  | ERS672246  | n.a. | CFV/CFVI   |
| Cff | 2008/170h | Human | feces  | FR  | ERS672247  | n.a. | CFV/CFVI   |
| Code  | Year/ID   | Source     | Type                | Accession | Notes |
|-------|-----------|------------|---------------------|-----------|-------|
| Cff   | 2008/568h | Human      | joint fluid        | FR        | ERS672248 | n.a     |
| Cff   | 2008/604h | Human      | feces               | FR        | ERS672249 | n.a     |
| Cff   | 2008/691h | Human      | cerebrospinal fluid| FR        | ERS672250 | n.a     |
| Cff   | 2008/755h | Human      | blood               | FR        | ERS672251 | n.a     |
| Cff   | 2010/41h  | Human      | feces               | FR        | ERS672253 | n.a     |
| Cff   | 2010/524h | Human      | kidney              | FR        | ERS672254 | n.a     |
| Cff   | 2010/1094h| Human      | blood               | FR        | ERS672255 | n.a     |
| Cff   | 2010/1119h| Human      | feces               | FR        | ERS672256 | n.a     |
| Cff   | 2010/1180h| Human      | blood               | FR        | ERS672257 | n.a     |
| Cff   | 2012/60h  | Human      | feces               | FR        | ERS672258 | n.a     |
| Cff   | 2012/185h | Human      | blood               | FR        | ERS672259 | n.a     |
| Cff   | 2012/286h | Human      | blood               | FR        | ERS672260 | n.a     |
| Cff   | 2012/331h | Human      | blood               | FR        | ERS672261 | n.a     |
| Cff   | 2012/879h | Human      | feces               | FR        | ERS672263 | n.a     |
| Cff   | 2012/1045h| Human      | joint fluid        | FR        | ERS672264 | n.a     |
| Cff   | 2014/52h  | Human      | cerebrospinal fluid| FR        | ERS672265 | n.a     |
| Cff   | 2014/602h | Human      | blood               | FR        | ERS672266 | n.a     |
| Cff   | 2014/790h | Human      | blood               | FR        | ERS672267 | n.a     |
| Cff   | 2014/947h | Human      | blood               | FR        | ERS672269 | n.a     |
| Cff   | 2014/1097h| Human      | feces               | FR        | ERS672270 | n.a     |
| Cff   | 2007/123h | Human      | cerebrospinal fluid| FR        | ERS672271 | n.a     |
| Cff   | 2009/56h  | Human      | cerebrospinal fluid| FR        | ERS672272 | n.a     |
| Cff   | CF156     | Human      | blood               | TR        | ERS672273 | n.a     |
| Cfvi  | 21-C0091-10-14_2 | Bovine | prepuce | UK | ERS672276 | n.a |
| Cff   | GTC _08732| Human      | cerebrospinal fluid| JP        | ERS672218 | n.a     |
| Cff   | GTC _11236| Human      | feces               | JP        | ERS672220 | n.a     |
| Cff   | 96-48     | Human      | feces               | JP        | ERS672224 | n.a     |
| Cff   | Human      | JP          | ERS672226 | n.a     | CFF/CFVI |
|-------|------------|-------------|-----------|---------|----------|
| 01-187| blood      | JP          | ERS672226 | n.a     | CFF/CFVI |
| 2004/103h | cerebrospinal fluid | FR          | ERS672233 | n.a     | CFF/CFVI |
| 2004/199h | cerebrospinal fluid | FR          | ERS672234 | n.a     | CFF/CFVI |
| 2004/359h | blood      | FR          | ERS672235 | n.a     | CFF/CFVI |
| 2004/362h | placenta   | FR          | ERS672236 | n.a     | CFF/CFVI |
| 2004/526h | feces      | FR          | ERS672237 | n.a     | CFF/CFVI |
| 2004/598h | blood      | FR          | ERS672238 | n.a     | CFF/CFVI |
| 2004/605h | feces      | FR          | ERS672239 | n.a     | CFF/CFVI |
| 2004/637h | joint fluid | FR          | ERS672240 | n.a     | CFF/CFVI |
| 2006/222h | blood      | FR          | ERS672241 | n.a     | CFF/CFVI |
| ID111063 | blood      | CA          | ERS739225 | n.a     | CFF/CFVI |
| ID117228 | blood      | CA          | ERS739226 | n.a     | CFF/CFVI |
| ID129038 | blood      | CA          | ERS739227 | n.a     | CFF/CFVI |
| ID131159 | feces      | CA          | ERS739228 | n.a     | CFF/CFVI |
| ID134381 | feces      | CA          | ERS739229 | n.a     | CFF/CFVI |
| ID136207 | blood      | CA          | ERS739230 | n.a     | CFF/CFVI |
| ID136551 | blood      | CA          | ERS739231 | n.a     | CFF/CFVI |
| ID136656 | blood      | CA          | ERS739232 | n.a     | CFF/CFVI |
| ID136706 | blood      | CA          | ERS739233 | n.a     | CFF/CFVI |
| ID132939 | blood      | CA          | ERS739234 | n.a     | CFF/CFVI |
| 2975   | blood      | TW          | ERS739256 | n.a     | CFF/CFVI |
| 923    | blood      | TW          | ERS739257 | n.a     | CFF/CFVI |
| 7035   | blood      | TW          | ERS739258 | n.a     | CFF/CFVI |
| My5726 | blood      | TW          | ERS739259 | n.a     | CFF/CFVI |
| 1592   | blood      | TW          | ERS739260 | n.a     | CFF/CFVI |
| 1830   | blood      | TW          | ERS739261 | n.a     | CFF/CFVI |
| 8468   | blood      | TW          | ERS739262 | n.a     | CFF/CFVI |
| 0003304-2 | blood      | TW          | ERS739263 | n.a     | CFF/CFVI |
| 2115   | blood      | TW          | ERS739264 | n.a     | CFF/CFVI |
| 2819   | blood      | TW          | ERS739265 | n.a     | CFF/CFVI |
| 5871   | blood      | TW          | ERS739266 | n.a     | CFF/CFVI |
| 1666   | blood      | TW          | ERS739267 | n.a     | CFF/CFVI |
| Code | Code | Species | Tissue/Area | Type | Accession | Notes |
|------|------|---------|-------------|------|-----------|-------|
| Cff  | 9502 | Human   | blood      | TW   | ERS739270 | n.a   |
| Cfv  | 800  | Human   | blood      | TW   | ERS739271 | n.a   |
| Cff  | 8031708 | Human   | blood      | TW   | ERS739272 | n.a   |
| Cff  | 8025552 | Human   | blood      | TW   | ERS739273 | n.a   |
| Cff  | 3069482 | Human   | blood      | TW   | ERS739274 | n.a   |
| Cfv  | C1   | Bovine  | prepuce    | SP   | ERS739275 | n.a   |
| Cfv  | C2   | Bovine  | prepuce    | SP   | ERS739276 | n.a   |
| Cff  | C3   | Bovine  | prepuce    | SP   | ERS739277 | n.a   |
| Cff  | C4   | Bovine  | prepuce    | SP   | ERS739278 | n.a   |
| Cff  | C5   | Bovine  | prepuce    | SP   | ERS739279 | n.a   |
| Cfv  | C6   | Bovine  | prepuce    | SP   | ERS739280 | n.a   |
| Cff  | C7   | Bovine  | prepuce    | SP   | ERS739281 | n.a   |
| Cff  | C8   | Bovine  | prepuce    | SP   | ERS739282 | n.a   |
| Cff  | C11  | Bovine  | prepuce    | SP   | ERS739285 | n.a   |
| Cfvi| C12  | Bovine  | prepuce    | SP   | ERS739286 | n.a   |
| Cff  | C13  | Bovine  | prepuce    | SP   | ERS739287 | n.a   |
| Cff  | C14  | Bovine  | prepuce    | SP   | ERS739288 | n.a   |
| Cff  | C15  | Bovine  | prepuce    | SP   | ERS739289 | n.a   |
| Cff  | C16  | Bovine  | prepuce    | SP   | ERS739290 | n.a   |
| Cff  | C17  | Bovine  | prepuce    | SP   | ERS739291 | n.a   |
| Cff  | C19  | Bovine  | prepuce    | SP   | ERS739292 | n.a   |
| Cff  | C20  | Bovine  | prepuce    | SP   | ERS739294 | n.a   |
| Cff  | C21  | Bovine  | prepuce    | SP   | ERS739295 | n.a   |
| Cfv  | C22  | Bovine  | prepuce    | SP   | ERS739296 | n.a   |
| Cfv  | C23  | Bovine  | prepuce    | SP   | ERS739297 | n.a   |
| Cfv  | C24  | Bovine  | prepuce    | SP   | ERS739298 | n.a   |
| Cfv  | C25  | Bovine  | prepuce    | SP   | ERS739299 | n.a   |
| Cfvi| C26  | Bovine  | prepuce    | SP   | ERS739300 | n.a   |
| Cfv  | C27  | Bovine  | prepuce    | SP   | ERS739301 | n.a   |
| Cfvi| C28  | Bovine  | prepuce    | SP   | ERS739302 | n.a   |
| Cff  | C29  | Bovine  | prepuce    | SP   | ERS739303 | n.a   |
| Cfv  | C30  | Bovine  | prepuce    | SP   | ERS739304 | n.a   |
| Cfvi| C31  | Bovine  | prepuce    | SP   | ERS739305 | n.a   |
| Cfvi| C32  | Bovine  | prepuce    | SP   | ERS739306 | n.a   |
| Cfv | C33  | Bovine prepuce | SP | ERS739307 | n.a | CFF/CFVI |
|-----|------|----------------|----|-----------|-----|----------|
| Cfv | C34  | Bovine prepuce | SP | ERS739308 | n.a | CFF/CFVI |
| Cfv | BS 201/02 | Bovine prepuce | GE | ERS686632 | n.a | CFV |
| Cfv | BS 76/04 | Bovine foetus   | GE | ERS686633 | n.a | CFV |
| Cfv | BS 38/06 | Bovine prepuce | GE | ERS686634 | n.a | CFV |
| Cfv | 07BS020 | Bovine prepuce | GE | ERS686635 | n.a | CFV |
| Cfv | 08CS0024 | Bovine prepuce | GE | ERS686636 | n.a | CFF/CFVI |
| Cfv | 09CS0030 | Bovine prepuce | GE | ERS686637 | n.a | CFV |
| Cfv | 11CS0190 | Bovine prepuce | GE | ERS686638 | n.a | CFV |
| Cfv | 11CS0191 | Bovine prepuce | GE | ERS686639 | n.a | CFV |
| Cfv | 13CS0183 | Bovine prepuce | GE | ERS686640 | n.a | CFV |
| Cfv | 14CS0001 | Bovine prepuce | GE | ERS686641 | n.a | CFV |
| Cff | BS 456/99 | Ovine foetus | GE | ERS686642 | n.a | CFF/CFVI |
| Cff | BS 458/99 | Bovine foetus   | GE | ERS686643 | n.a | CFF/CFVI |
| Cff | BS 03/04 | Bovine foetus   | GE | ERS686644 | n.a | CFF/CFVI |
| Cff | BS 91/05 | Bovine prepuce | GE | ERS686645 | n.a | CFF/CFVI |
| Cff | 08CS0027 | Bovine prepuce | GE | ERS686646 | n.a | CFF/CFVI |
| Cff | 11CS0098 | Ovine placenta | GE | ERS686648 | n.a | CFF/CFVI |
| Cff | 12CS0302 | Bovine prepuce | GE | ERS686649 | n.a | CFF/CFVI |
| Cff | 13CS0001 | Bovine prepuce | GE | ERS686650 | n.a | CFF/CFVI |
| Cff | 13CS0373 | Monkey faeces | GE | ERS686651 | n.a | CFF/CFVI |
| Cff | 001A-0374 | Human blood | CA | ERS686652 | n.a | CFF/CFVI |
| Cff | 001A-0648 | Human blood | CA | ERS686653 | n.a | CFF/CFVI |
| Cff | LR133  | Ovine foetus   | NZ | ERS846544 | n.a | CFF/CFVI |
| Cff | 1      | Bovine prepuce | UK | ERS846553 | n.a | CFF/CFVI |
| Cff | 2      | Bovine prepuce | UK | ERS846554 | n.a | CFF/CFVI |
| Cff | 3      | Ovine placenta | UK | ERS846555 | n.a | CFF/CFVI |
| Cff | 4      | Ovine placenta | UK | ERS846556 | n.a | CFF/CFVI |
| Cff | 5      | Ovine placenta | UK | ERS846557 | n.a | CFF/CFVI |
| Cff | 6      | Bovine prepuce | UK | ERS846558 | n.a | CFF/CFVI |
| Cff | 7      | Ovine foetus   | UK | ERS846559 | n.a | CFF/CFVI |
| Cff | 8      | Ovine foetus   | UK | ERS846560 | n.a | CFF/CFVI |
| Cff | 9      | Ovine placenta | UK | ERS846561 | n.a | CFF/CFVI |
| Cff | 12     | Ovine placenta | UK | ERS846562 | n.a | CFF/CFVI |
| Cff | 13 | Bovine | prepuce | UK     | ERS846563 | n.a   | CFF/CFVI |
|-----|----|--------|---------|--------|-----------|-------|----------|
| Cff | 14 | Ovine  | placenta | UK     | ERS846564 | n.a   | CFF/CFVI |
| Cff | 15 | Ovine  | placenta | UK     | ERS846565 | n.a   | CFF/CFVI |
| Cff | 17 | Ovine  | foetus   | UK     | ERS846566 | n.a   | CFF/CFVI |
| Cfv | JCM_2528 | Bovine | vaginal mucus | UK     | ERS846567 | n.a   | CFF/CFVI |
| Cfv | 161/97 | Bovine | prepuce | BR     | ERS846568 | n.a   | CFF/CFVI |
| Cfv | 515/98 | Bovine | prepuce | BR     | ERS846569 | n.a   | CFF/CFVI |