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Veterinary Newsletter

April 2018

## **Feline Infectious Peritonitis**

### **Lessons from Luca: use a reliable veterinary laboratory**

#### **Key message: sensitivity is EXTREMELY IMPORTANT when using a FCoV antibody test to rule out FIP**

Luca was a cat with histopathologically confirmed feline infectious peritonitis (FIP). The purpose of this educational film was to emphasise the importance of sensitivity of a feline coronavirus (FCoV) antibody test when ruling out FIP as a diagnosis. As a consequence of using an insensitive test from Antech Diagnostics, Luca was dragged round a series of veterinarians in search of a diagnosis; he was subjected to numerous blood samples and his guardian suffered the distress of desperately trying to help her deteriorating cat, not to mention that she ran up a bill in four figures during the process.

Summary of the problems with the Antech Diagnostics FCoV antibody test:

1. It is mis-labelled FCV, which is the abbreviation for feline calicivirus
2. Most importantly, the first dilution of the sample is 1:400, i.e. they report any antibody titre below 1 in 400 as negative. The recommended starting dilution is from 1:8 or 1:10 to 1:25. <sup>Addie, 2012</sup>
3. Antech offers to confirm FIP by a 7b ELISA test which is based on a laboratory artefact.

#### **Key message: send the CORRECT sample for the test required:**

- **blood for FCoV antibody test, not effusion**
- **effusion for FCoV RT-PCR, not blood**

The second mistake Luca's veterinary surgeon made was sending blood for FCoV RT-PCR: the result was negative. Most cats with FIP will not have FCoV RNA in their blood; a fine needle aspirate of a lesion should have been sent in Luca's case, since he had non-effusive FIP. In effusive FIP cases, the effusion should be sent for FCoV RT-PCR testing.

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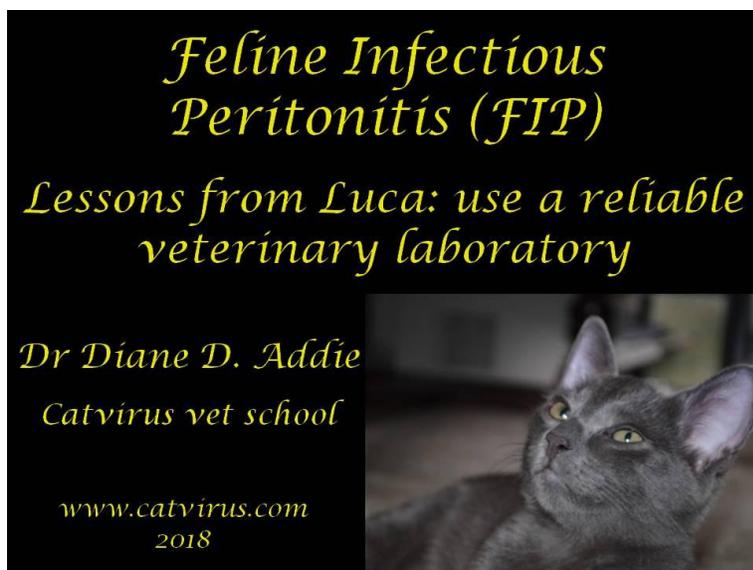
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To watch the video, use any of the following links and please like the video and become a follower / subscriber:

**D Tube:** <https://d.tube/#!/v/catvirus/aew9h026>

**Bitchute:** <https://www.bitchute.com/video/Hh5Mo3GYPKyp/>

**YouTube:** [https://youtu.be/p\\_V-Y405VZg](https://youtu.be/p_V-Y405VZg)

## Transcript of the narrative

This film is one of the catvirus veterinary school series: it is primarily for veterinary professionals but it does contain a message for cat guardians too.

Disclaimer: I accept no responsibility for any actions you may take as a consequence of watching this film: If you have a sick cat please consult a qualified veterinary surgeon.

This video is part of a series of videos entitled “Lessons from Luca” sponsored by Maria Bonino. Luca was a cat who had non-effusive FIP. As a consequence of a poor choice of external veterinary laboratory, he was subjected to many more blood samples than he required, and his guardian, Maria, ran up a huge bill in her quest to find out what was wrong with her beloved cat.

In this film, I am going to discuss step 4 of the non-effusive FIP diagnosis algorithm: that is to say, sending blood samples to an external veterinary laboratory.

You may wish to download and print out a copy of the non-effusive FIP diagnostic algorithm to have beside you during this film, if so please press pause and go to [www.catvirus.com](http://www.catvirus.com), click on the Downloads page: you will find the flowchart available in many languages, thanks to our heroic band of translators. [<http://www.catvirus.com/downloads.html>] The algorithm in English is usually the most up to date.

Lesson 1 is to use a GOOD veterinary laboratory, which knows about FIP.

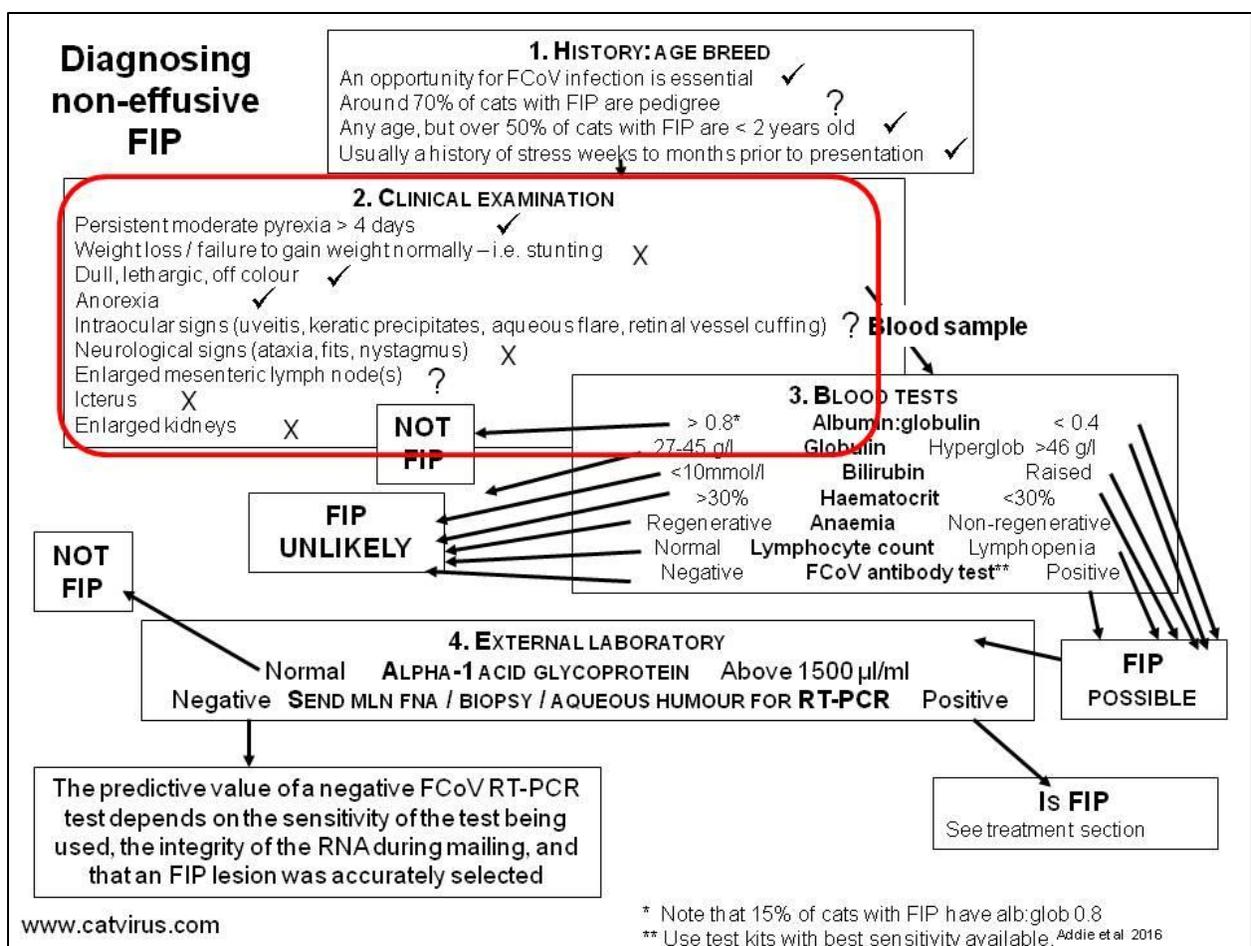
- Lesson 2 is to send the correct sample to the external laboratory and ask for the correct tests: send blood for FCoV antibody testing (not effusion)
- send effusion for FCoV RT-PCR testing (not blood)

History: Luca was rescued from from a Los Angeles rescue group on Sunday 23 September 2012: at age almost 9 weeks – he was already neutered

On Monday 9 September 2013, when Luca was 14 months old, his guardian, to use her words “Noticed that Luca was not as “enthusiastic” about his dinner as he normally was but he ate pretty much all of it so I didn’t think it was a big deal – maybe just a case of ‘upset stomach’.”

Maria took him to the first of many veterinary surgeons, and quite correctly, that veterinary surgeon included a test for FCoV antibodies in the initial screen. This video is about Step 4 of the FIP diagnostic algorithm: Steps 1 and 2 of the non-effusive FIP diagnostic flowchart for Luca will be dealt with in detail in other videos. However, I will go through them briefly: he had an opportunity to become infected with FCoV because he was from a rescue shelter, and he also lived in a house with 3 other cats. Was he a purebred cat? He could have been, he certainly looked like a Russian blue. He was 14 months old, which is obviously less than 2 years, and he had had a booster vaccination in the weeks coming up to his first clinical signs – so the stress question was also answered in the affirmative.

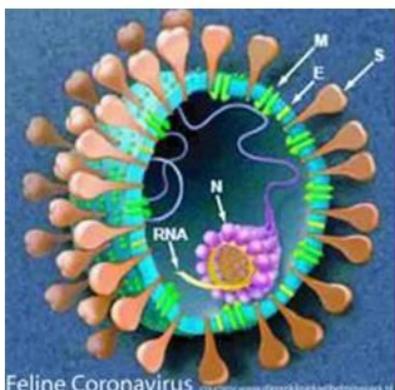
Step 2 of the algorithm – the clinical signs – were not so clear-cut: he ticked only 3 of the 9 parameters and those signs - anorexia, pyrexia and lethargy – are common to a huge number of feline diseases. I don’t know if the attending veterinarian checked for intraocular signs or an enlarged mesenteric lymph node, so I have put question marks there.



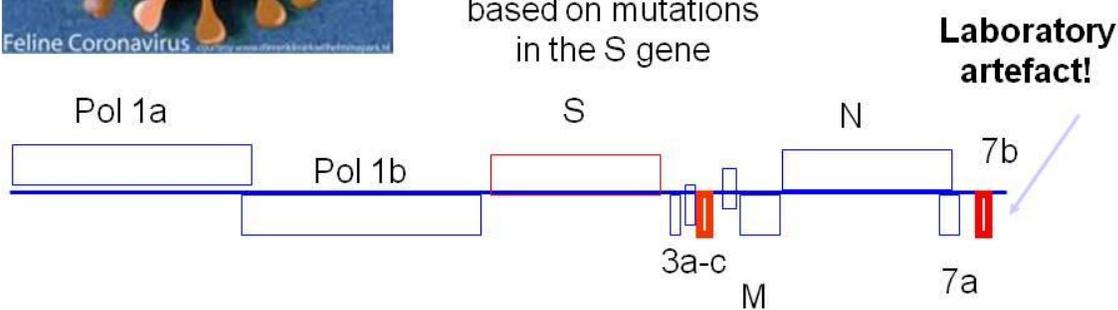
The attending vet then did absolutely the correct thing: he or she took blood samples and included a screen test for feline coronavirus and toxoplasma antibodies. The samples were not tested in-house – which would usually be Step 3 of the algorithm – they were sent to an external laboratory, in this case Antech. Antech was a very unfortunate choice of laboratory: let's have a look at the results. First, they have the wrong abbreviation for feline coronavirus: FCV is the internationally recognised abbreviation for feline calicivirus: the correct feline coronavirus abbreviation is FCoV. I pointed this out to Antech back in 2009 and received the reply that, essentially, they'd always done it that way and that to change it – i.e. correct it – would “confuse” their clients. Personally, I would be a lot more confused by a wrong abbreviation. I have confirmed that, as of 2018, they still use the wrong abbreviation.

The next problem was that the starting dilution of the sample was 1 in 400: this is far too big a dilution, most laboratories begin somewhere between 1 in 10 and 1 in 25: this will mean that Antech will report a lot of false negative results. We don't know if Luca had FCoV antibodies, because he wasn't tested elsewhere, but he did have histopathologically confirmed non-effusive FIP and it is close to inconceivable that he would have been FCoV antibody negative. So I cannot say this result was a false negative, but I strongly suspect that it was, and the consequence was that Luca's veterinarians hunted around for a diagnosis other than FIP, wasting valuable time that could have been used to treat him, putting him through un-necessary tests and costing his guardian a fortune.

## Deletions were found in the 7b and 3c FCoV genes



The Idexx test is based on mutations in the S gene



Herrewegh AAPM., Vennema H., Horzinek MC., Rottier PJM, de Groot RJ. 1995 The molecular genetics of feline coronaviruses: comparative sequence analysis of the ORF7a/7b transcription unit of different biotypes. *Virology*. 212:622-631

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The third thing wrong about this test was that they offered a 7b ELISA test to “confirm FIP diagnosis.” To be fair – I’m not sure that they are still doing this – but when Luca was ill, they were and at that time the 7b test had been long discredited. <sup>Herrewegh *et al*, 1995; Lin *et al*, 2009</sup> The tale is that molecular biologists sequencing laboratory strains noticed a deletion in the 7b gene [called 6b at the time] in laboratory strains called Feline Enteric Coronavirus – FECV, but that the 7b gene was intact in FIP laboratory strains. <sup>Vennema *et al*, 1992</sup> [You can read more about it here: <http://www.catvirus.com/FCoVantibody.htm#7BELISA>] That was itself strange – it would have meant that an insertion would be needed for FIP to develop, rather than a mutation or a deletion, which are much more common. Turned out the 7b deletion was a laboratory artefact: if you grow FCoV in cell culture it doesn’t need an intact 7b gene so mutants with deletions there can grow happily. <sup>Herrewegh *et al*, 1995</sup> FCoV is an RNA virus: it mutates, deletes and recombines all the time, that’s what RNA viruses do. Anyway, a test was developed based on this laboratory artefact. Dr Melissa Kennedy published a paper which evaluated the 7b antibody test and she concluded her abstract by saying “Seropositivity for this protein [i.e. 7b] was not specific for ... a diagnosis of FIP.” <sup>Kennedy *et al*, 2008</sup>

Some years ago I invited Antech to take part in a study of FCoV antibody tests but they declined. The results of that study were published in JFMS and you can see which tests performed best on the FCoV antibody page of my [www.catvirus.com](http://www.catvirus.com) website.

To find that page, go to the [catvirus.com](http://www.catvirus.com) website, choose the What Is FIP? page, scroll down till you find the FCoV antibody link [<http://www.catvirus.com/FCoVantibody.htm>] and when you press that it’ll come up in a new tab. The contents of the page are listed at the top: the page describes the uses of FCoV antibody tests, and how to interpret the results, there are a list of FCoV antibody tests which are available and whether or not I recommend them, and if you scroll down to the foot of the page you’ll find the list of veterinary laboratories that I recommend: this list is not exhaustive, it’s only the ones I know about personally, so if you are a veterinary laboratory person and want to tell me about your own laboratory do feel free to contact me. The page might look a little different from the one shown here because it gets updated regularly. There is a direct link to the FCoV antibody page in the notes below. The test which performed best overall was the FCoV Immunocomb: this is a test that can be performed in a veterinary practice by any reasonably competent person, so you wouldn’t need access to an external veterinary laboratory. You can contact Len Small to find out how to obtain it in your country. The best performing rapid immunomigration test was the Speed F-Corona from Virbac.

Now this is where I have a message for any cat guardians who might still be listening – and have not been bored to death thus far: YOU are paying for the laboratory test and you have the right to ask that your cat’s sample be sent to a laboratory that knows about FCoV and FIP.

OK – back to Luca’s story: on Saturday 14<sup>th</sup> September 2013 Luca was given an ultrasound at a hospital: the veterinarian on duty speculated that he had FIP. The veterinary surgeon sent samples for an RT-PCR test. Guess where they were sent – yup, Antech. And once again Antech gave a negative RT-PCR result for Luca – a cat proven to have had FIP. “Well how can that be?” you are probably asking yourself. Simple – the wrong sample was sent: Luca didn’t have an effusion, so a blood sample was sent. Do NOT send blood for FCoV RT-PCR testing! EVER! Even if the laboratory asks you to! RT-PCR on a blood sample is not useful: most cats with FIP are negative, as Luca was, and some cats without FIP can be transiently positive. To be fair to Antech, they are not alone in offering this test, there are even veterinary schools, who should know better, doing exactly the same: for example, North Carolina, seen here, and Lisbon, Portugal. I used to work with the legendary veterinary orthopaedic surgeon Malcolm Ness, and he had a charming expression for procedures which he deemed useless, he would say: “You might as well piss in its ear!” Not so easy when you’re a female veterinarian! I think he’d use that expression for sending a blood sample for FCoV RT-PCR!

Laboratory	Antech	Antech	Antech	Doctor Y's Hospital	Idexx	Idexx	Antech	Idexx
Date	11 Sep 2013	13-Sep-13	15-Sep-13	16-Sep-13	20-Sep-13	25-Sep-13	03-Oct-13	04-Oct-13
Day post FIP presentation	0	2	4	5	9	14	21	23
Hct	42	36		31.2	25.5	20.2	24	
MCV	40	38		40	38.2	37.8	43	
Lymphocytes	1194	1248		980	530	680	1140	
Alb	2.9				2.5	2.1	2.5	1.8
Glob	6.6				4.5	3.8	3.1	4
A:G	0.44					0.5	0.8	0.5
Bilirubin	0.3				0.4	1.4	2.5	3.1
FCoV Ab	< 1:400							
Toxo	Negative							
FCoV mRNA PCR			Negative					

This is the table I made of all the laboratory results of Luca, which Maria Bonino kindly sent to me. You can see that his Hct was declining sharply over the 24 days of his disease: I’ll be discussing this more in another video. MCV would have increased if he’d been making new red blood cells: he wasn’t: he had the non-regenerative anaemia of chronic disease that we commonly see in FIP. And here I just have to have another go at Antech – look at the results on October 3<sup>rd</sup>, sandwiched between two results from Idexx – and all I can say is REALLY? REALLY? For that day only, Luca’s Hct increased, his lymphocytes jumped up and his albumin:globulin ratio became 0.8 – a level which rules out FIP – on the day before he died of FIP. REALLY? This begs the question of whether you can trust ANY results from that laboratory, not just the FCoV / FIP results.

To finish off, let us summarise the Lessons From Luca we have covered today:

First: use a GOOD veterinary laboratory that knows about FIP: if you are a cat guardian, YOU will be paying for the test and have the right to ask for your cat’s sample to be sent to a reputable laboratory

A list of recommended laboratories can be found at [www.catvirus.com/FCoVantibody.htm](http://www.catvirus.com/FCoVantibody.htm) and the link is in the notes below this video.

Second, once you’ve chosen a good laboratory, Get the RIGHT tests done:

- i.e. FCoV antibody test on blood
- (not effusion) and
- RT-PCR on effusion, not blood

I give my deepest gratitude to Maria Bonino for allowing Luca’s story to be used to help others, for supplying his laboratory results and for sponsoring this video. Maria is a platinum catvirus

subscriber and this video honours the memory of Luca and its purpose is to try to prevent other cats from going through what he went through. Finally, many thanks to Mike at coastal launch services for his gorgeous voiceover at the end of this video.

If you found this video useful, please like it, share it, become a subscriber to my channel and website.

Thank you very much for your attention.

God bless you and your cats.

## Acknowledgements

I give my deepest gratitude to Maria Bonino for allowing Luca's story to be used to help others, for supplying his laboratory results and for sponsoring this video.

Huge thanks to François Bagaïni of [www.vetocyte.fr](http://www.vetocyte.fr) for the catvirus animation at the end of the video and many thanks to Mike Edgerton of Coastal Launch Services for doing the catvirus logo voiceover.

[www.coastallaunchservicesltd.co.uk](http://www.coastallaunchservicesltd.co.uk)

I want to give a BIG thank you to [www.online-convert.com](http://www.online-convert.com) for converting video and audio – none of my films would be possible without them. I also thank the generous programmers of Audacity.

I am also very grateful to Blaine for supplying a version of Windows Movie Maker: again, this video would not have been possible with that resource.

<http://movies.blainesville.com/2010/05/installing-windows-movie-maker-60-on.html>

## Further information

1. FCoV antibody page direct link: <http://www.catvirus.com/FCoVantibody.htm>
2. To obtain the FCoV Immunocomb, contact Len Small: [len@biogal.co.il](mailto:len@biogal.co.il)
3. Please support me in making more education videos by becoming a subscriber at <http://www.catvirus.com/index.htm#subscribe>
4. Check out my book "FIP and Coronavirus" here: [https://www.amazon.com/Feline-Infectious-Peritonitis-Coronavirus-Everything/dp/1480208973/ref=tmm\\_pap\\_swatch\\_0?encoding=UTF8&qid=1522678318&sr=8-1-fkmr0](https://www.amazon.com/Feline-Infectious-Peritonitis-Coronavirus-Everything/dp/1480208973/ref=tmm_pap_swatch_0?encoding=UTF8&qid=1522678318&sr=8-1-fkmr0)
5. My D Tube channel: <https://d.tube#!/c/catvirus>
6. My Bitchute channel: <https://www.bitchute.com/channel/ZEk9qdH9iDzm/>
7. My YouTube channel: <http://www.youtube.com/user/DrDianeDAddie>
8. My blogs can be found on Steemit: <https://steemit.com/@catvirus>
9. Follow me on Twitter @FIPvet - <https://twitter.com/FIPvet>
10. Get healthy - eat more vegetables: find my recipes in "Protein Rich Soup" book [https://www.amazon.com/Protein-Rich-Soup-Where-Your/dp/197580175X/ref=sr\\_1\\_2?s=books&ie=UTF8&qid=1522683182&sr=1-2&keywords=protein+rich+soup](https://www.amazon.com/Protein-Rich-Soup-Where-Your/dp/197580175X/ref=sr_1_2?s=books&ie=UTF8&qid=1522683182&sr=1-2&keywords=protein+rich+soup)
11. Facebook: [www.facebook.com#!/profile.php?id=100000644732948](http://www.facebook.com#!/profile.php?id=100000644732948)

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