SUPPLEMENTARY DATA

Relative sensitivity of immunohistochemistry, multiple reaction monitoring mass spectrometry, *in situ* hybridization and PCR to detect Coxsackievirus B1 in A549 cells

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Additional information about study design and methods

Proteomics

The final MRM assay conditions used in the study were: ion spray voltage of 2800 V, curtain gas, 20 psi; Nebulizer gas 10, and interface heater temperature of 125°C. Collision energy 22.3; Dwell

time 100 msec. LC conditions, Buffer A; 0.1% formic acid/water, Buffer B; 0.1% formic acid/acetonitrile; Column, PicoFrit (75 μM inner diameter, 2 μM tip opening, New Objective, Woburn, MA) resin 10 cm of reverse phase 5 μM, 100 Å Magic C18 resin (Michrom Bioresources, Auburn, CA). Flow rate, 500nl/min; gradient 5–10% Solvent B in 3 min, 10–60% solvent B in 48 min, and 60–95% solvent B for 5 min before re-equilibration with 95% solvent A for 7 min.

Immunohistochemistry

In house antibodies against the four capsid proteins (VP1-VP4) of CVB4 virus (GenBank database – accession no. DQ480420):

The antibodies were raised in rabbits as follows: cloned recombinant VP1, VP2, VP3 and VP4 fused with His-tag (VP1-His, VP2-His, VP3-His, and VP4-His) were expressed in E.Coli, VP-His proteins were purified employing nickel columns and used for rabbit immunization. Two laboratories (Exeter and Tampere) optimized these antibodies for their IHC techniques using EV-infected cells in FFPE cell arrays. The final dilutions which were selected based on optimization are shown in Supplementary Table 1.

Supplementary Table 1. Concentrations of the CVB4 VP1-VP4 antibodies used in the IHC stainings in Exeter and Tampere.

Laboratory	**VP1A**	**VP1B**	**VP2B**	**VP3A**	**VP3B**	**VP4B**
Exeter (UK)	1:3000	1:6000	1:3000	1:2500	1:9000	1:2000
Tampere (FIN)	1:3000	1:4000	1:3200	1:1500	1:5000	1:2500

Additional information for the Results

Supplementary Table 2. The RT-PCR results of different dilutions of CVB1 infected A549 cells

Sample dilutions	**real-time PCR Tampere (FIN) CT-value**	**semi-nested PCR Uppsala (SWE)**
Undiluted	13,6	positive
10^{-1}	14,3	positive
10^{-2}	16,2	positive
10^{-3}	18,9	positive
10^{-4}	23,7	positive
10^{-5}	26,2	positive
10^{-6}	30,6	positive
10^{-7}	40,6	positive
10^{-8}	negative	positive
Negative control[1]	negative	negative

[1] Uninfected A549 cells treated in the same way as infected cells