

Is EBV DNA detectable in CSF in multiple sclerosis?

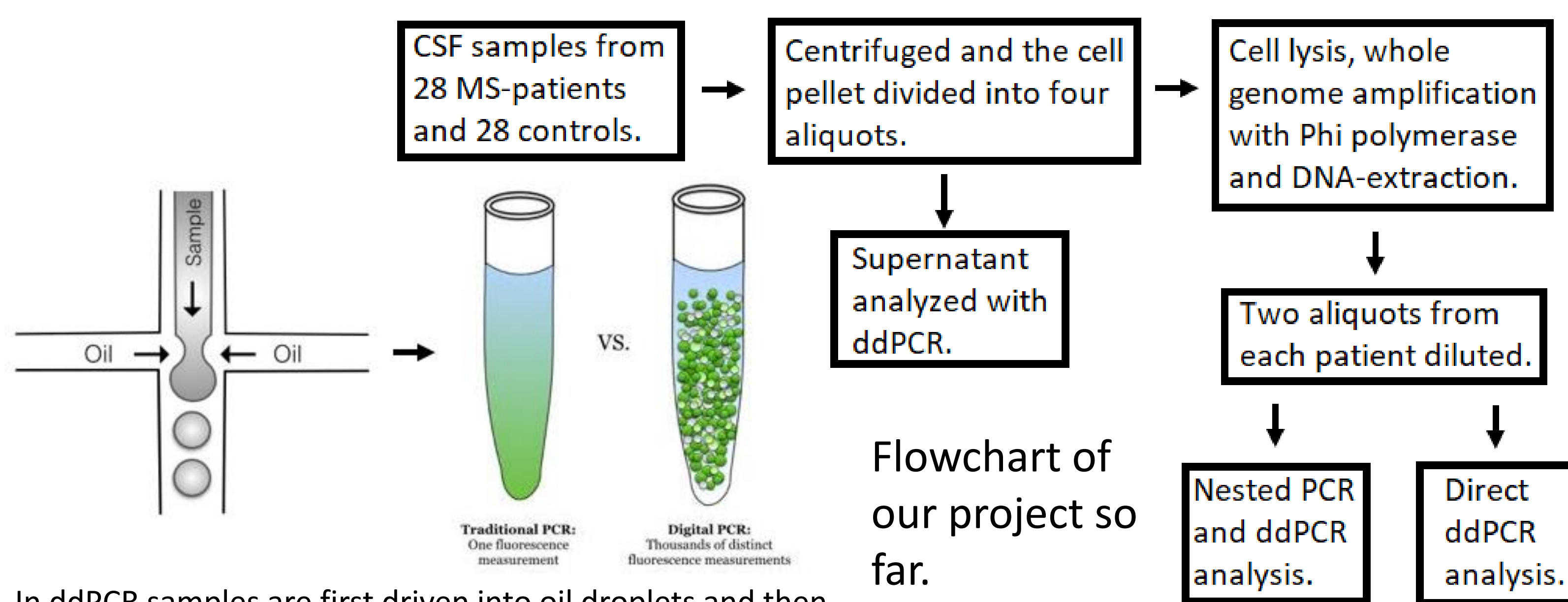
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Introduction

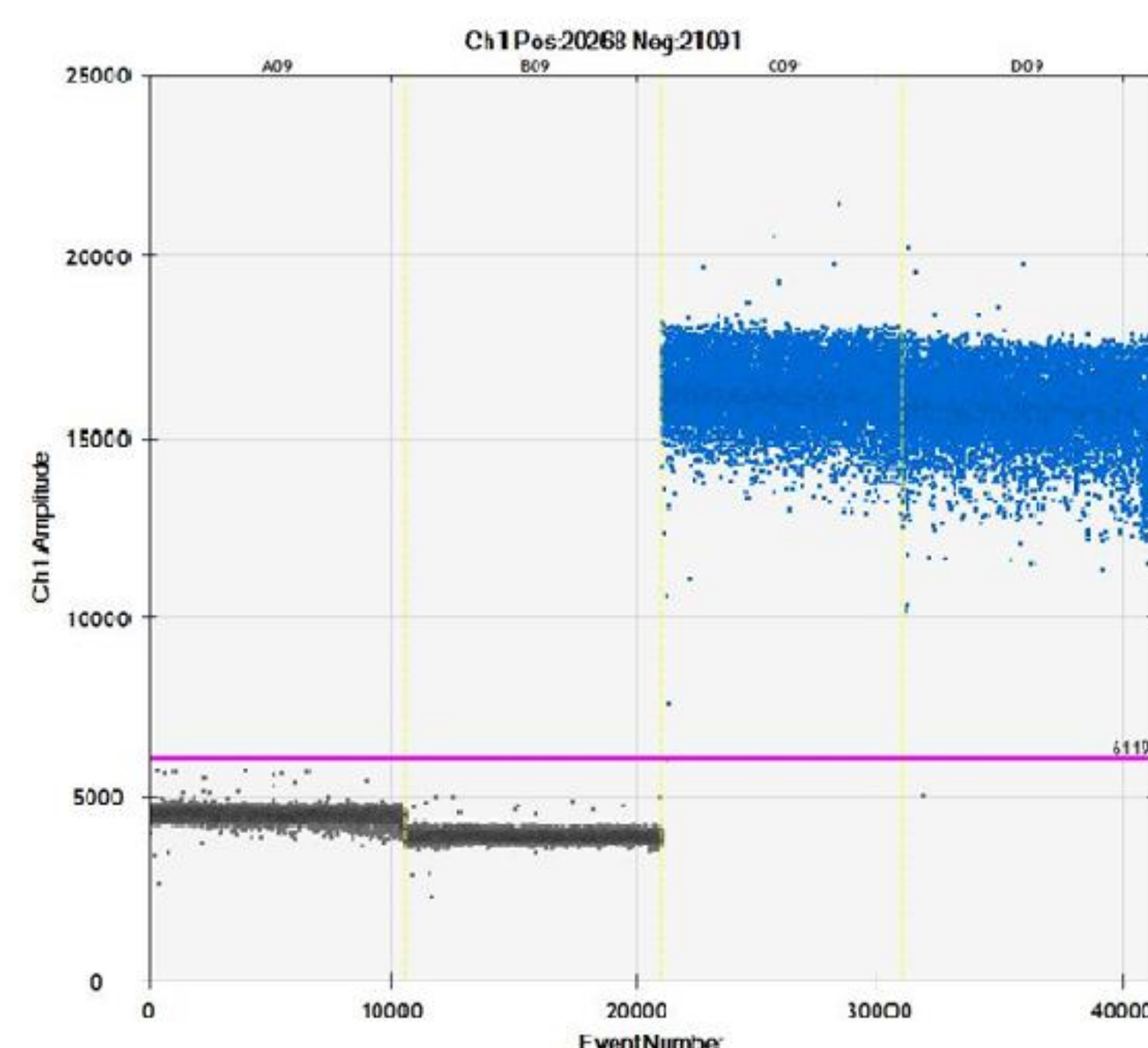
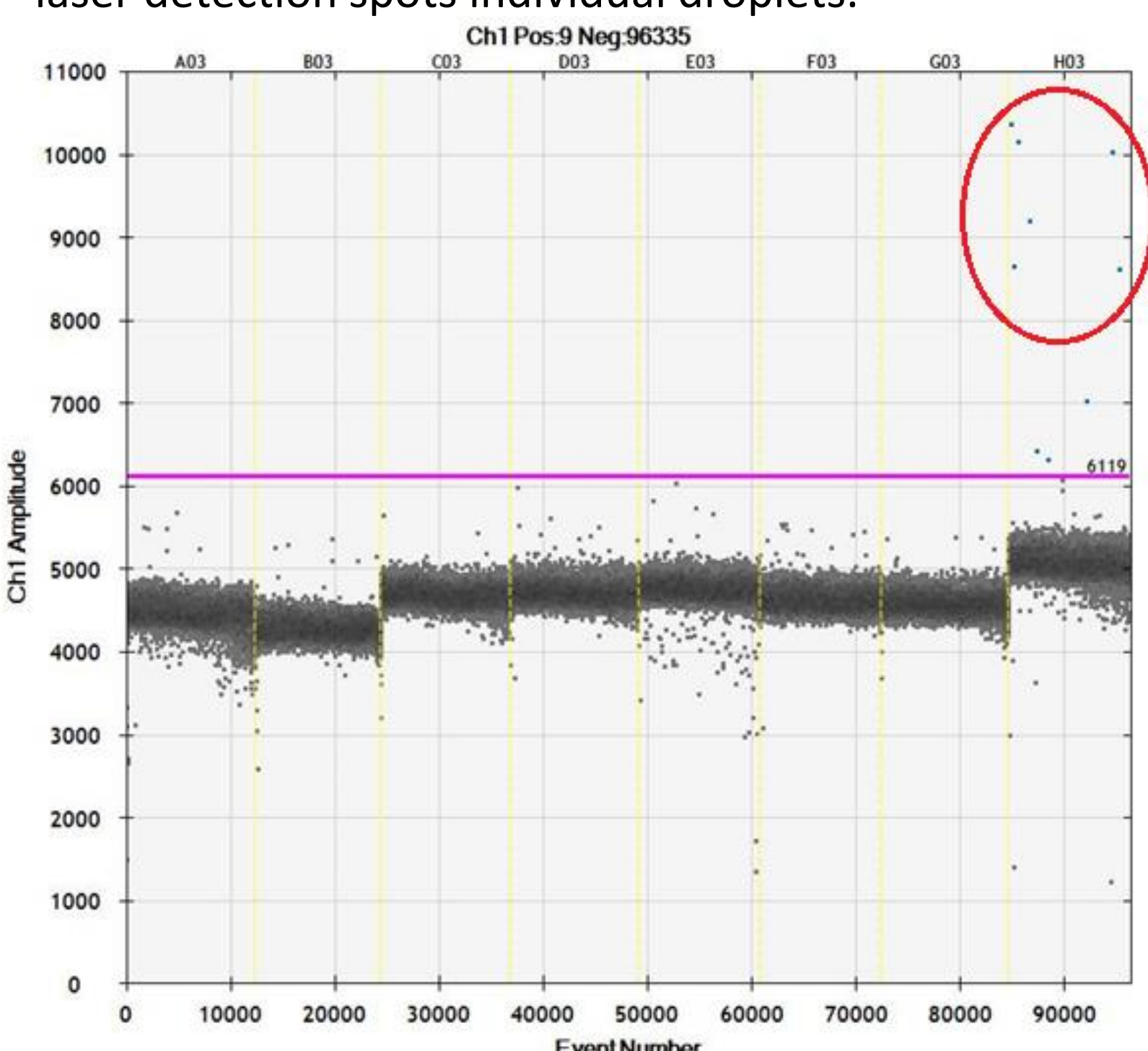
- Epstein-Barr Virus (EBV) is an oncovirus which causes mild childhood infection or mononucleosis postpubertally as the primary infection and can form latency that lasts for a lifetime.
- EBV causes Burkitt's and Hodgkin lymphoma and has also been associated with nasopharyngeal and gastric carcinoma, SLE, RA and MS.
- New studies have reported that close to 100 % of multiple sclerosis (MS) (vs. 95% controls) patients have EBV antibodies. ⁽¹⁾
- Causation or correlation? Causation would mean that MS is a rare complication of late EBV infection. Possible mechanisms:
 - EBV-activation in the CNS B-cells leads to CD8 + attack and by-stander demyelination. ⁽²⁾
 - EBV activation in the periphery leads to CD8+ activation and migration to the CNS and demyelination via molecular mimicry of T-cell selfpeptides between CNS and EBV. ⁽³⁾
 - Immunomodulatory effects of EBV on B-cells, T-cells and other cells. ⁽⁴⁾
- There are contradictory findings about the presence of EBV in the CNS of MS patients. ⁽⁵⁾

Methods

- CSF samples collected at diagnostic lumbar punctures, 28 patients subsequently diagnosed with MS and 28 controls with other conditions.
- Samples were centrifuged, live cells aliquoted and cellular DNA was amplified (Phi polymerase).
- Sensitive Digital droplet PCR (ddPCR) was used.
- Cellular DNA: Two aliquots of amplified DNA were used for viral DNA detection and two EBV DNA detection methods were applied (in total 4 tests per patient sample):
 - direct ddPCR of diluted sample.
 - dilution, PCR to amplify EBV target followed by nested ddPCR.
- CSF supernatant: DNA was extracted (1 ml) and analysed by direct ddPCR.
- PCR and ddPCR target region was within a repetitive sequence in EBV genome. ddPCR data analysis was done with Bio-Rad QuantaSoft™.
- Sample dilution was done because amplified DNA used as such inhibited the fluorescent reaction and nested PCR reactants inhibited droplet formation.



In ddPCR samples are first driven into oil droplets and then PCR is performed. Analysis is then performed, computerized laser detection spots individual droplets.



Charts showing an example of part of a ddPCR run; on the left 8 patient samples with 1 showing some positive droplets (blue dots above the purple line [inside the red circle]), on the right negative and positive controls (amplified DNA from EBV-positive T-cells).

Results

- CSF cellular DNA: 6 MS patients (21%) and 5 controls (18%) showed positivity in one of the 4 tests. (p=n.s.)
- CSF supernatants: One MS patient (3,5%) was positive for EBV DNA.

Patientcode	ddPCR 1. aliquot		ddPCR 2. aliquot		nested-ddPCR 1. aliquot		nested-ddPCR 2. aliquot	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
C14	1		1		1			1
C15	1		1		1			1
C22		1	1		1		1	
C27	1			1	1		1	
C8	1		1		1			1
MS1	1		1			1	1	
MS13	1		1			1	1	
MS26	1			1	1		1	
MS27	1			1	1		1	
MS6		1	1		1		1	
MS7	1		1		1			1

Table showing MS-patients (MS#) and controls (C#) who had EBV-DNA positivity in one of the tests.

Discussion

- Our findings are in line with previous studies in that EBV DNA can be found in CSF of both MS patients and controls.
- Problem with our results is that no MS patient or control had more than one positive result in the four tests performed.
- The possible explanations for this include the low amount of EBV DNA in samples (<10 ng) and that the general DNA amplification might not have replicated viral DNA in all samples as prominently.
- However, the fact that EBV DNA is detectable shows that the EBV has access to CNS both in MS patients and controls.
- We will continue by purifying the amplified DNA samples and running ddPCR and HERQ9-PCR to verify the results.

Conclusions

- EBV can be detected from both the CSF of MS patients and controls.
- The amount of EBV-DNA in CSF seems to be very small.
- There doesn't seem to be a mechanism prohibiting access of all EBV positive cells to the CNS.
- We will continue by purifying the amplified DNA samples and running ddPCR and HERQ9-PCR* to verify the results with about 100 ng of sample DNA per reaction (previously <10ng).

*) HERQ9 = a novel multiplex quantitative PCR to differentiate and quantify all nine human herpes viruses.

References

- S. Abrahamyan, B. Eberspächer, M-M. Hoshi et al: Complete Epstein-Barr virus seropositivity in a large cohort of patients with early multiple sclerosis, *J Neurol Neurosurg Psychiatry* (doi:10.1136/jnnp-2020-32294).
- J.S. Haring, L.L. Pewe, S. Perlman: Bystander CD8 T cell-mediated demyelination after viral infection of the central nervous system, *J. Immunol.* 169 (2002) 1550–1555.
- K. W. Wucherpfennig, J. L. Strominger: Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein, *Cell* 1995 Mar 10;80(5):695-705.
- J. B. Harley et al.: Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity, *Nat Genet.* 2018 May;50(5):699-707.
- B. Serafini, B. Rosicarelli, C. Veroni, G. A. Mazzola, F. Aloisi: CD8 T cell response to EBV in multiple sclerosis, *J. Virology online* 2.10.2019.
- M. P. Pender, P. A. Csurhes, C. M. M. Pfluger et al.: CD8 T cell deficiency impairs control of Epstein-Barr virus and worsens with age in multiple sclerosis, *J Neurol Neurosurg Psychiatry* 2012;83:353-354.
- J. O. Virtanen et al: Oligoclonal bands in multiple sclerosis reactive against two herpesviruses and association with magnetic resonance imaging findings, *Mult Scler.* 2014 Jan;20(1):27-34.
- E. Kuusela et al: Serum Epstein-Barr virus DNA, detected by droplet digital PCR, correlates with disease activity in patients with rheumatoid arthritis, *Clinical and Experimental Rheumatology* 2018.
- L. Pyörä et al: HERQ-9 Is a new multiplex PCR for differentiation and quantification of all nine human herpesviruses, *mSphere* :e00265-20.

Pictures of ddPCR:
https://commons.wikimedia.org/wiki/File:Droplet_Formation_in_ddPCR.jpg
https://commons.wikimedia.org/wiki/File:DdPCR_vs_Traditional_PCR.jpg