Neurovirology

Assays for the detection of human polyomavirus DNA in blood, CSF, and brain tissue at the National Institutes of Health

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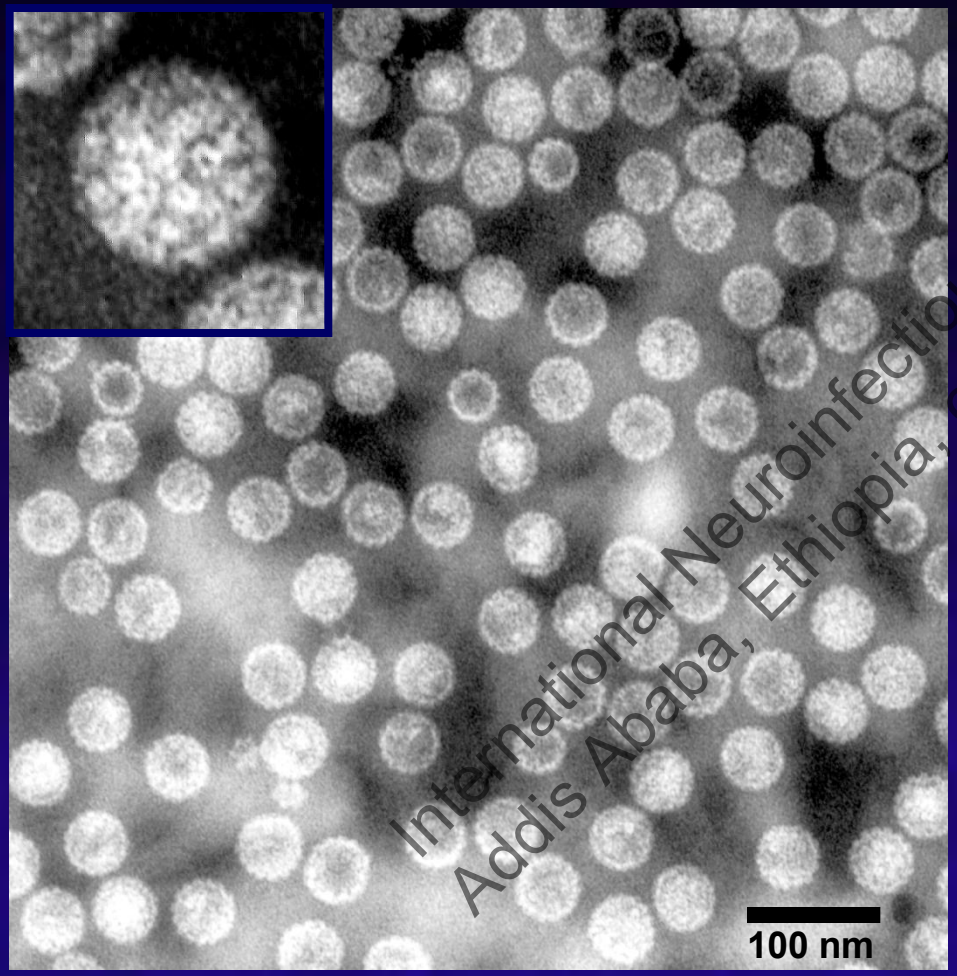
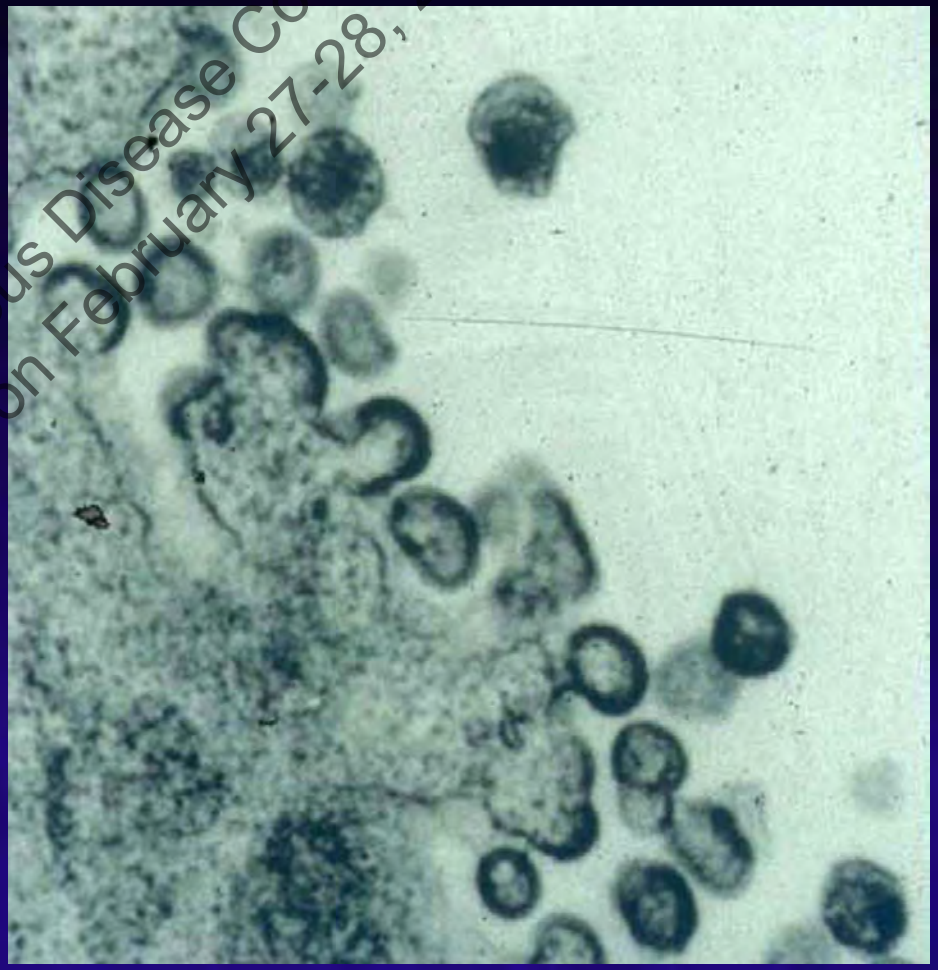
Neurotropic Viruses

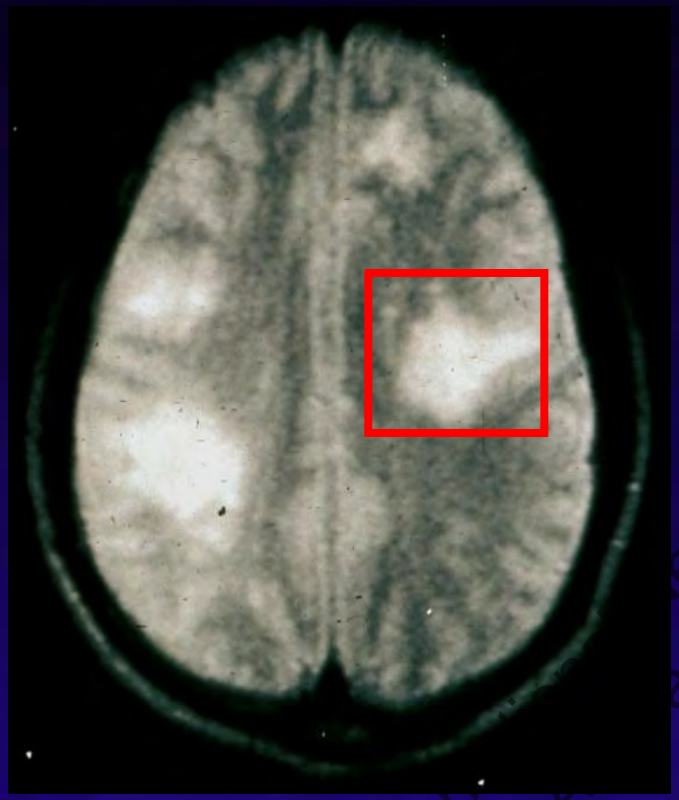
JCV/PML

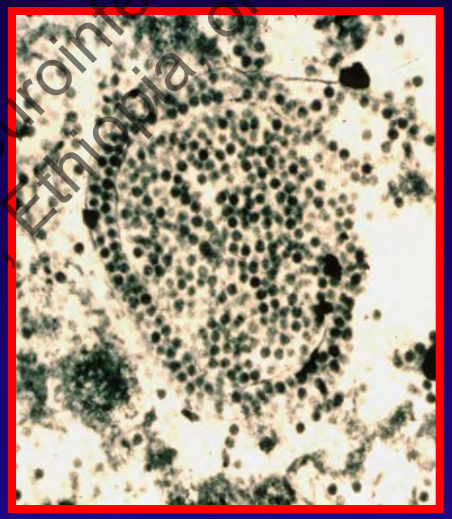


HIV-1/AIDS



Progressive Multifocal Leukoencephalopathy





Clinical Symptoms

- Cognitive impairments
- Visual deficits
- Motor dysfunction

** Language disturbances, seizures and headaches more frequent in AIDS patients*

PML occurrence

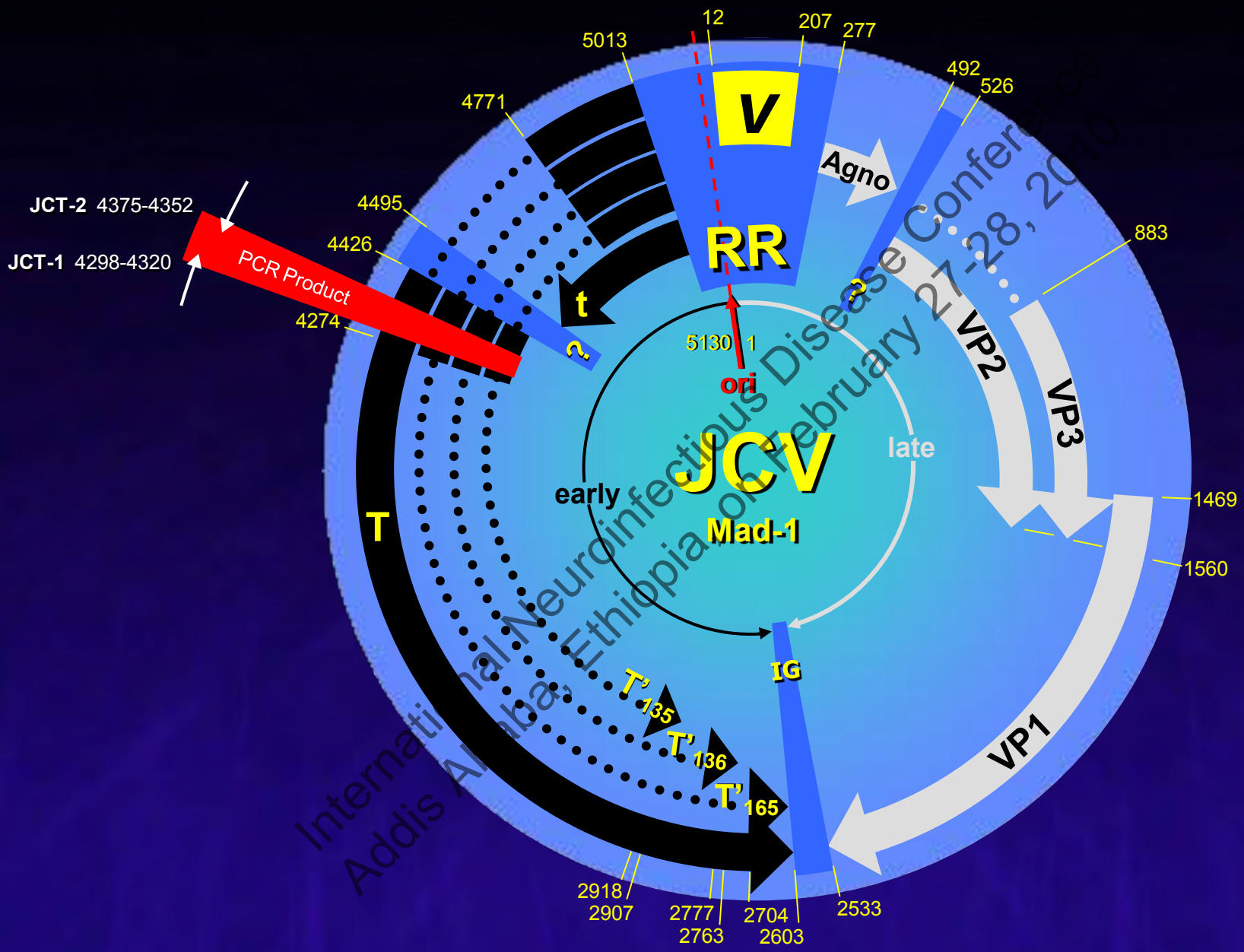
Immune compromised host

3-5% in HIV-1+ (global), AIDS defining illness

Not always fatal with immune reconstitution i.e. IRIS, 'plex'

High antibody titers to JCV, not protective(?)

Immune surveillance/protection by CD8+ cytotoxic T cells directed to epitopes on VP1



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

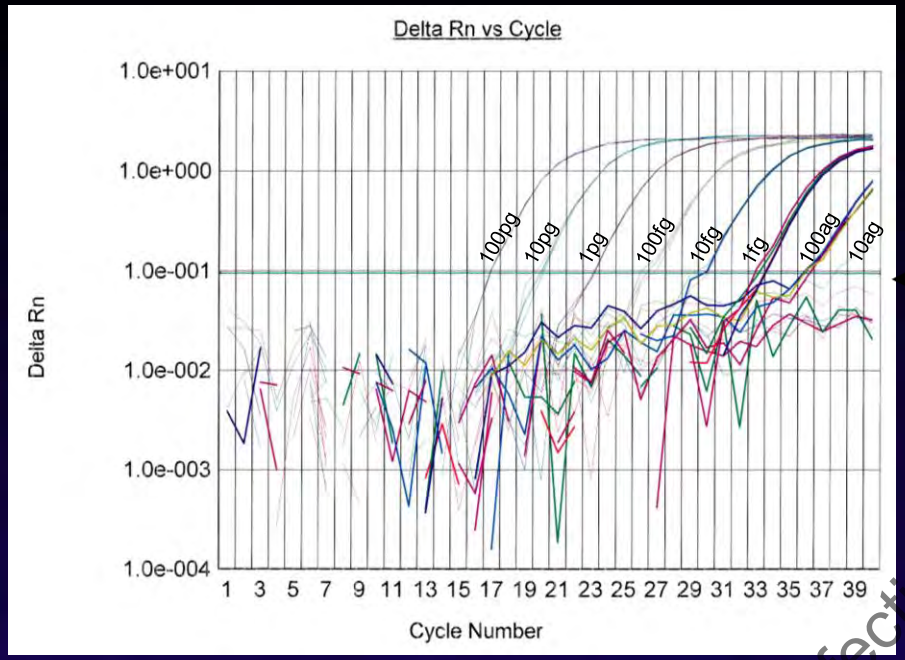
National Institutes of Health

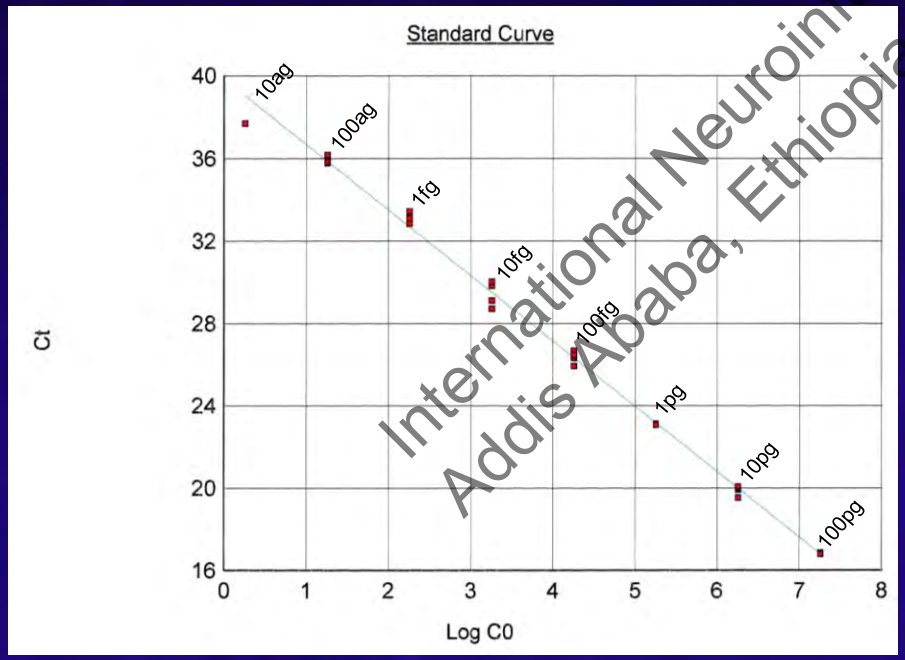
qPCR Assay for Detection of JC Virus

Description of Invention: JC Virus causes a fatal disease in the brain called progressive multifocal leukoencephalopathy (PML) that occurs in many patients with immunocompromised conditions. For example, more than five percent (5%) of AIDS patients develop PML. Additionally, these conditions include, but are not limited to, cancers such as leukemias and lymphomas, organ transplants such as kidney, heart and autoimmune conditions with treatment that modulates the immune system such as Multiple Sclerosis (MS), rheumatoid arthritis, psoriasis, and systemic lupus erythematosus. The finding of JCV DNA in the patients with neurological symptoms of PML is a diagnostic criterion and is needed to confirm the diagnosis of PML to rule out other neurological conditions.

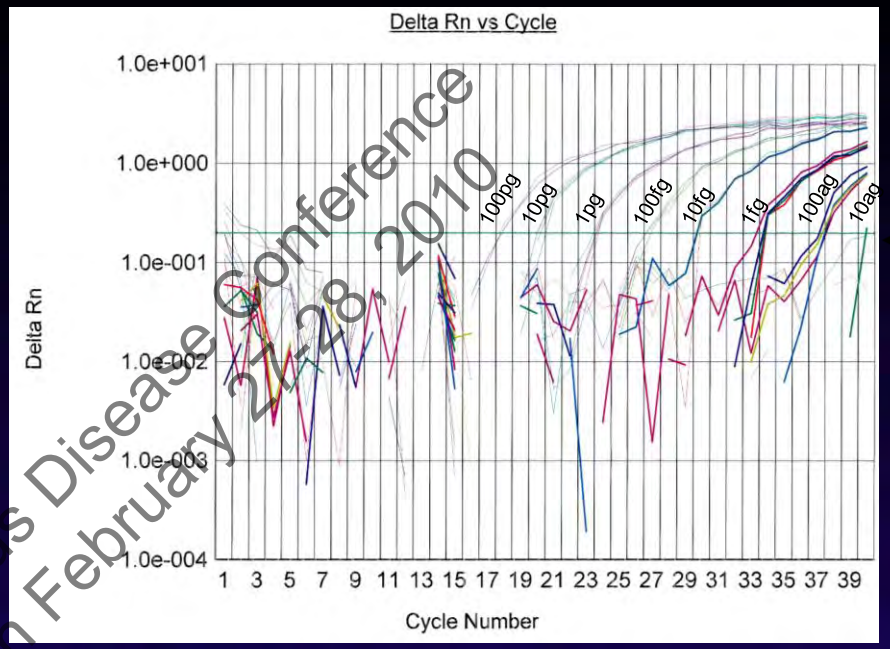
This technology describes a qPCR assay that utilizes viral DNA standards and testing samples to detect the presence of the JC viral genome in patients' cerebrospinal fluid and blood, blood products, and tissue samples from biopsy or autopsy.

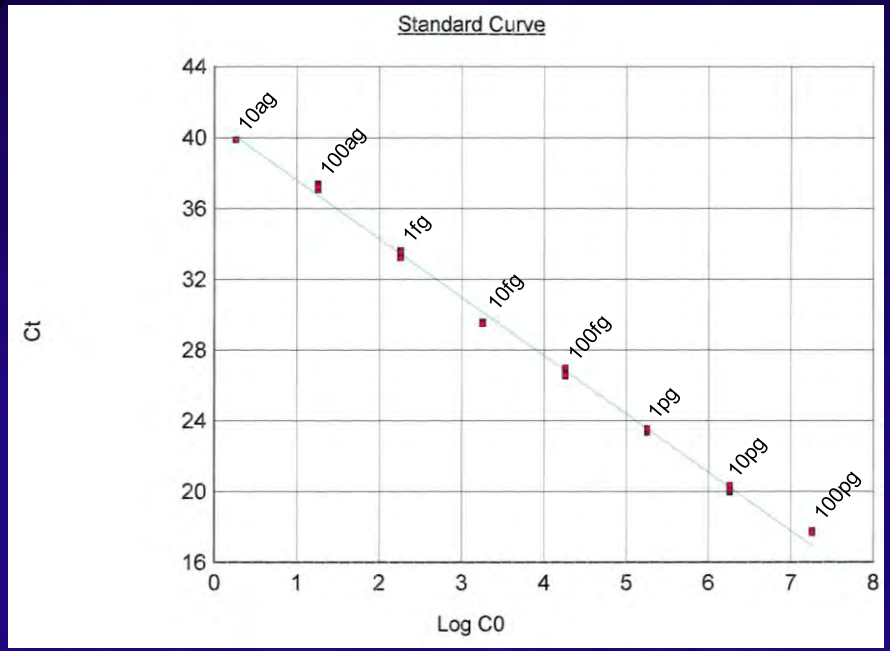
JCV





BKV





Development of Progressive Multifocal Leucoencephalopathy (PML) in a 37 year old male with Multiple Sclerosis treated with Natalizumab (Tysabri®) monotherapy

2008	28.1	26.5	18.6	27.6	9.7	11.7	28.7	7.8	14.8	15.8	21.8	27.8	28.8	3.9	10.9	23.9	7.10	11.11
Treatm		Nat 15		Nat 16		PLEX 11/7 - 21/7			Mirt				IVMP 28/8-30/8				Ifn-β 20/10	
time from PLEX							1 week		4 weeks		5 weeks			7 weeks				
Nat, µg/mL						11/7: 14.4	16/7: 8.7	18/7: 5.5										
Diagnosis					9/7 PML				14/8 IRIS									
Sympt	0	Myoclonias left arm and hand from 26/4																
				Weakness left arm and hand														
							Leftsided hemiparesis											
									Fever, headache, fatigue from 10/8									
											Relapse; vertigo, nausea							
														Improvement				
EDSS	1.0	1.0	1.0	2.0	2.0	2.0	3.5	3.5	7.5	7.5	8.0	8.0	8.0	7.5	7.5	7.5	7.0	6.0

Development of Progressive Multifocal Leucoencephalopathy (PML) in a 37 year old male with Multiple Sclerosis treated with Natalizumab (Tysabri®) monotherapy

MRI	28.1	26/5	18.6		9.7		28.7	7.8	14.8		21.8	27.8		3.9	10.9	23.9	7.10	11.11
MC	+	+	+		+		+	+	+		+	+		nec	nec	nec	nec	nec
IC							+	+	+		+	+		+	+	+	+	+
BG								+	+		+	+		+	+	+	+	+
Vol, mL	<1	1	3		5		12	35	40		45	45		45	45	45	45	45
Gad, +/-	-	-	-		-		-	-	+		++	+++		++	++	++	+	(+)
CSF			19.6			11.7		7.8		15.8			28.8					
MNC			0			2		2		82			20					
albumin			122			126		158		234			457					
JCV																		
SMI			<200			<200		<200		<200			<200					
NIH			-			53		10		Ud			Ud					

Treatm; treatment, *Nat*; Natalizumab, *PLEX*; plasma exchange, *Mirt*; mirtazapin, *IVMP*; intravenous methylprednisolon, *Ifn-β*; intramuscular interferon-beta 1a, *Nat, µg/mL*; concentrations of Natalizumab in blood before the first, third and fourth PLEX treatment. *IRIS*; immune reconstitution inflammatory syndrome, *Sympt*; symptoms, EDSS; expanded disability status scale, *MRI*; magnetic resonance imaging, *MC*; high signal lesion in motor cortex, *nec*; cortical laminar necrotosis in motorcortex, *IC*; high signal lesion internal capsule, *BG*; high signal lesion basal ganglia, *Vol*; MRI lesion volume, *Gad*; gadolinium enhancement, *CSF*; cerebrospinal fluid, *MNC*; mononuclear cells (x 10^9/L), *Albumin* (mg/L), JCV; JC virus, *SMI*; JCV polychain reaction (PCR) (copies/mL) at Smittskyddsinstitutet, Stockholm, Sweden, *NIH*; JCV PCR (copies/mL) at NIH, Bethesda, USA. Ud; undetectable

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Publications:

1. ML Landry et al. False negative PCR despite high levels of JC virus DNA in spinal fluid: Implications for diagnostic testing. J Clin Virol. 2008 Oct;43(2):247-249. [PubMed abs]

2. C Ryschkewitsch et al. Comparison of PCR-southern hybridization and quantitative real-time PCR for the detection of JC and BK viral nucleotide sequences in urine and cerebrospinal fluid. J Virol Methods. 2004 Nov;121(2):217-221. [PubMed abs]

3. T Yousry et al. Evaluation of patients treated with natalizumab for progressive multifocal leukoencephalopathy. N Engl J Med. 2006 Mar 2;354(9):924-933. [PubMed abs]

Patent Status: HHS Reference No. E-152-2009/0 – Research Material. Patent protection is not being pursued for this technology.

Licensing Status: Available for licensing.

Licensing Contact: Peter A. Soukas, J.D., 301-435-4646; soukasp@mail.nih.gov

Table 1. PCR Sensitivity Dependence on DNA Template Preparation

	Control CSF Samples*	Lab A — Robotic Extraction# (500 µl)^	Lab B — Manual Extraction# (200 µl)	Lab B Re-tests; Lab A Extract
copies/ml	7.2E+10	3.90E+08	3.50E+09	3.80E+08
	7.2E+09	2.60E+07	5.00E+08	6.40E+07
	7.2E+08	3.60E+06	5.75E+07	6.00E+06
	7.2E+07	5.00E+05	5.75E+06	5.30E+05
	7.2E+06	5.30E+04	5.25E+05	5.70E+04
	7.2E+05	6.19E+03	5.00E+04	6.34E+03
	7.2E+04	990	6.48E+03	1.12E+03
	7.2E+03	130	1.03E+03	50

* Pooled negative CSF spiked with cloned JCV (whole genome).
# Robotic extraction using commerical MagNA Pure instrument (Roche); manual extraction using spin column concentration QIA amp kit (Quiagen).
^ Starting volume of sample.

Comparison of JC Virus extraction methods

QIAamp Viral RNA Kit #52904	QIAamp MinElute Virus Kit #57704
1. 200 µl sample extracted	1. 200 µl sample extracted
2. 8 µg/reaction carrier RNA/AVL buffer made and held at 4 C	2. 6.2 µg/reaction carrier RNA/AL buffer made fresh daily
3. AVL (Viral lysis buffer) contains only chaotropic salt, no protease enzyme	3. AL lysis buffer plus protease helps to lyse some viral DNA btter than chaotropic salt alone
4. Spin column made to elute 50 µl or more	4. Minielute spin column specificially designed to elute smaller volumes, 20 µl to 150 µl. Results in more concentrated template
5. 50 µl eluted	5. 25 µl eluted
6. 3600 copies JC plasmid DNA spiked into 1mL of plasma and extracted. Real time PCR results: 475 copies/mL (13% recovery)	6. 3600 copies JC plasmid DNA spiked into 1mL plasma and extracted. Real time PCR results: 1566 copies/mL (44% recovery)

Note: Carrier RNA provided in kit improves binding of viral DNA to Qiamp membrane, especially for low-titer samples and limits possible degradation of the DNA.

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The QCMD programme is organised in collaboration with the European Society for Clinical Virology and the European Society for Clinical Microbiology & Infectious Diseases.

Final Report

QCMD 2007
JC virus and BK virus (JCBKDNA07)
Pilot study

Paola Cinque, William G MacKay &
Anton M van Loon
on behalf of QCMD and its Scientific Advisory Board
August 2007

Program Details

JCBKDNA07	
Date of panel distribution	20/03/2007
Number of participants	79
Number of countries	26
Number of respondents	71 (90%)
Number of datasets submitted	128 *
Number of qualitative datasets submitted	44 (34%)
Number of qualitative and quantitative datasets submitted	84 (66%)
Combined datsets	
Number of datasets submitted	22
Number of qualitative datasets submitted	13 (59%)
Number of qualitative and quantitative datasets submitted	9 (41%)
JCV-only datasets	
Number of datasets submitted	53
Number of qualitative datasets submitted	19 (36%)
Number of qualitative and quantitative datasets submitted	34 (64%)
BKV-only datasets	
Number of datasets submitted	53
Number of qualitative datasets submitted	12 (23%)
Number of qualitative and quantitative datasets submitted	41 (77%)

Panel Composition

Sample	Sample content *	Sample matrix *	Target CT value	Sample status *
JC.BK07-01	BKV-2/JCV-1	VTM	30 / 30	W. Pos / Str. Pos
JC.BK07-02	JCV-1	VTM	28 - 30	Strong Positive
JC.BK07-03	JCV-1	VTM	38 - 40	Weak Positive
JC.BK07-04	JCV-1	VTM	34 - 37	Positive
JC.BK07-05	JCV-2	VTM	40 - 45	Weak Positive
JC.BK07-06	JCV-2	VTM	34 - 37	Positive
JC.BK07-07	JCV-2	VTM	31 - 33	Positive
JC.BK07-08	JCV-1	Plasma	31 - 33	Positive
JC.BK07-09	Negative	VTM		Negative
JC.BK07-10	JCV-2	VTM	38 - 40	Weak Positive
JC.BK07-11	BKV-1	VTM	28 - 30	Strong Positive
JC.BK07-12	BKV-1	VTM	34 - 37	Positive
JC.BK07-13	BKV-2	VTM	40 - 45	Weak Positive
JC.BK07-14	BKV-2	VTM	34 - 37	Positive
JC.BK07-15	BKV-1	VTM	38 - 40	Weak Positive
JC.BK07-16	BKV-2	VTM	38 - 40	Weak Positive
JC.BK07-17	BKV-2	Plasma	31 - 33	Positive
JC.BK07-18	BKV-1	VTM	31 - 33	Positive
JC.BK07-19	BKV-2/JCV-1	VTM	30 / 38	Str. Pos / W. Pos
JC.BK07-20	Negative	VTM		Negative

Samples	Total # Samples	Positive Samples/Total
Single CSF (Affirm/Sentinel)	392	0/392
Single Plasma (Affirm/Sentinel)	273	12/273 (4.4%)
Karolinska CSF study	401	0/401
		[2/614]
Karolinska Plasma study	342	3/342 (1%)
CSF	793	0/793
Plasma	615	15/615 (2.4%)

Plasma (antibody) and CSF Samples Tested

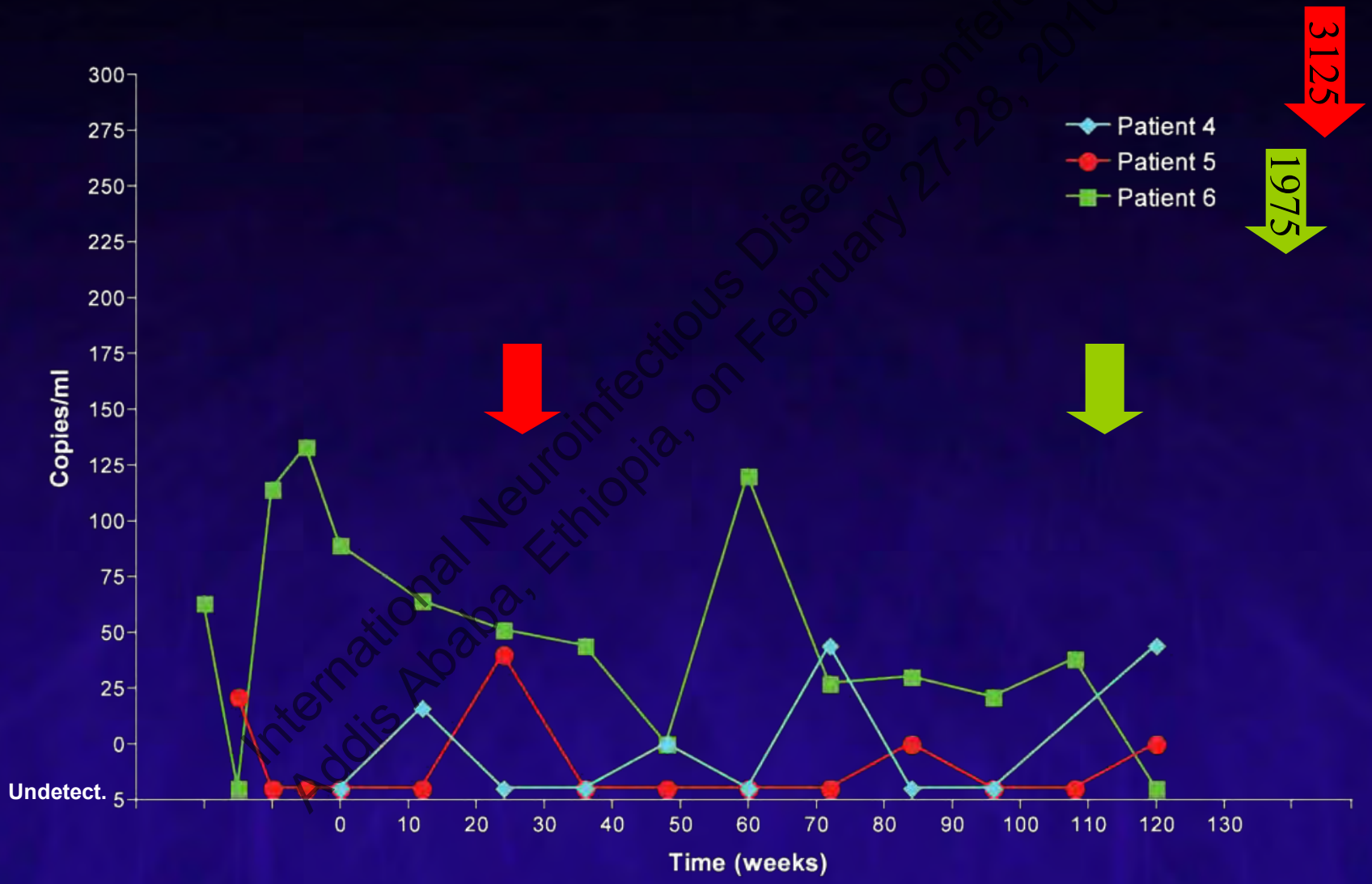
Country	No. Samples	<640	640-2560	10,240-40960	163,840 or >	CSF Samples Tested
Argentina	1	0	0	1	0	5
Australia	7	1	4	2	0	4
Austria	5	1	1	3	0	0
Belgium	1	1	0	0	0(1PML)	0
Canada	10	2	6	2	0	16
Czech Rep.	12	0	8	4	0	66
Denmark	1	0	1	0	0	0
Finland	1	0	1	0	0	0
France	27	8	15	4	0	0
Greece	0	0	0	0	0	4
Germany	28	7	11	10	0	8
Hungary	8	1	3	0	4	0
Israel	2	0	2	0	0	1
Italy	16	4	10	1	1	13
Netherlands	0	0	0	0	0	4
New Zealand	2	1	0	1	0	1
Norway	1	0	1	0	0	0
Poland	4	2	1	1	0	4
Slovak Rep	1	0	1	0	0	0
South Africa	0	0	0	0	0	6
Spain	13	3	8	2	0	34
Switzerland	2	1	1	0	0	2
Turkey	2	0	2	0	0	0
United Kingdom	2	1	1	0	0	8
USA	127	48	59	19 (1PML)	1 (1PML)	208 (2PML)
Totals	**273**	**81**	**136**	**50**	**6**	**388**
Lack location	0	0	0	0	0	4

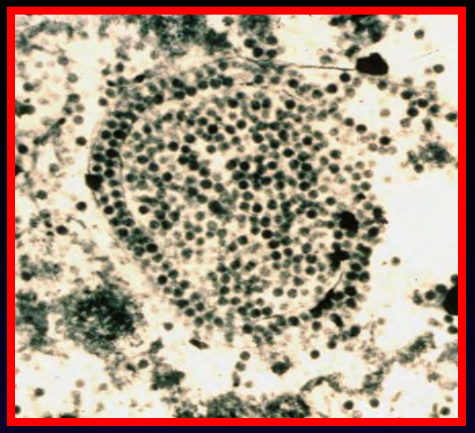
TABLE 2. JCV status of Plasma or CSF Samples from Patients in the AFFRIM/SENTINEL Trial

MS*	Crohn's	RA	NK	Total
ELISA ASSAY on Plasma: Antibody titers >640 (sero+); <640 (sero -)				
(214)	(40)	(5)	--	**(259)**
65% +	90% +	80% +	--	**69.9% +**
35% -	10% -	20% -	--	**30.1% -**
2560-10,240"	10,240-40,960"	10,240	--	
qPCR ASSAY on Plasma: Detection level at 25 genome copies/ml				
(224)	(40)	(5)	(1)	**(270)**
3.1%	12.5%	0%	0%	**4.4%**
7^/224	5^/40			**12/264**
116 Longitudinal samples from the 12 viremic patients				
29%	63%	--	--	**36%**
27/94	15/22	--	--	**42/116**
qPCR ASSAY on CSF: Detection level at 25 genome copies/ml				
(346)	(34)	(4)	(4)	**(388)**
0/346	0/34	0/4	0/4	**0/388**

" Average antibody titer in ELISA assay using 4x serial dilutions.
* MS patients treated with natalizumab: 208/224 (93%); 16/224 on Avonex (7%). Crohn's patients: 38/40 treated with natalizumab (95%); 2 Placebo. Patients in the AFFIRM and SENTINEL Phase III trials receiving one or more doses of drug.
^ 3/7 MS patients with a single detectable plasma sample only received Avonex. All 5 Crohn's patients were treated with natalizumab.
# A set of other longitudinal samples, from MS patients on natalizumab, were tested from 20 single samples previously screened by a commercial source whose laboratory did not find detectable viral DNA.
MS, multiple sclerosis; RA, rheumatoid arthritis; NK, diagnosis unknown.
-- Not applicable.

Quantitative PCR for viral DNA in longitudinal plasma samples:





qPCR assay parameters

*Genome location for primers at amino terminal end of T protein

*Template extraction critical for sensitivity ("reconstruction" tests)

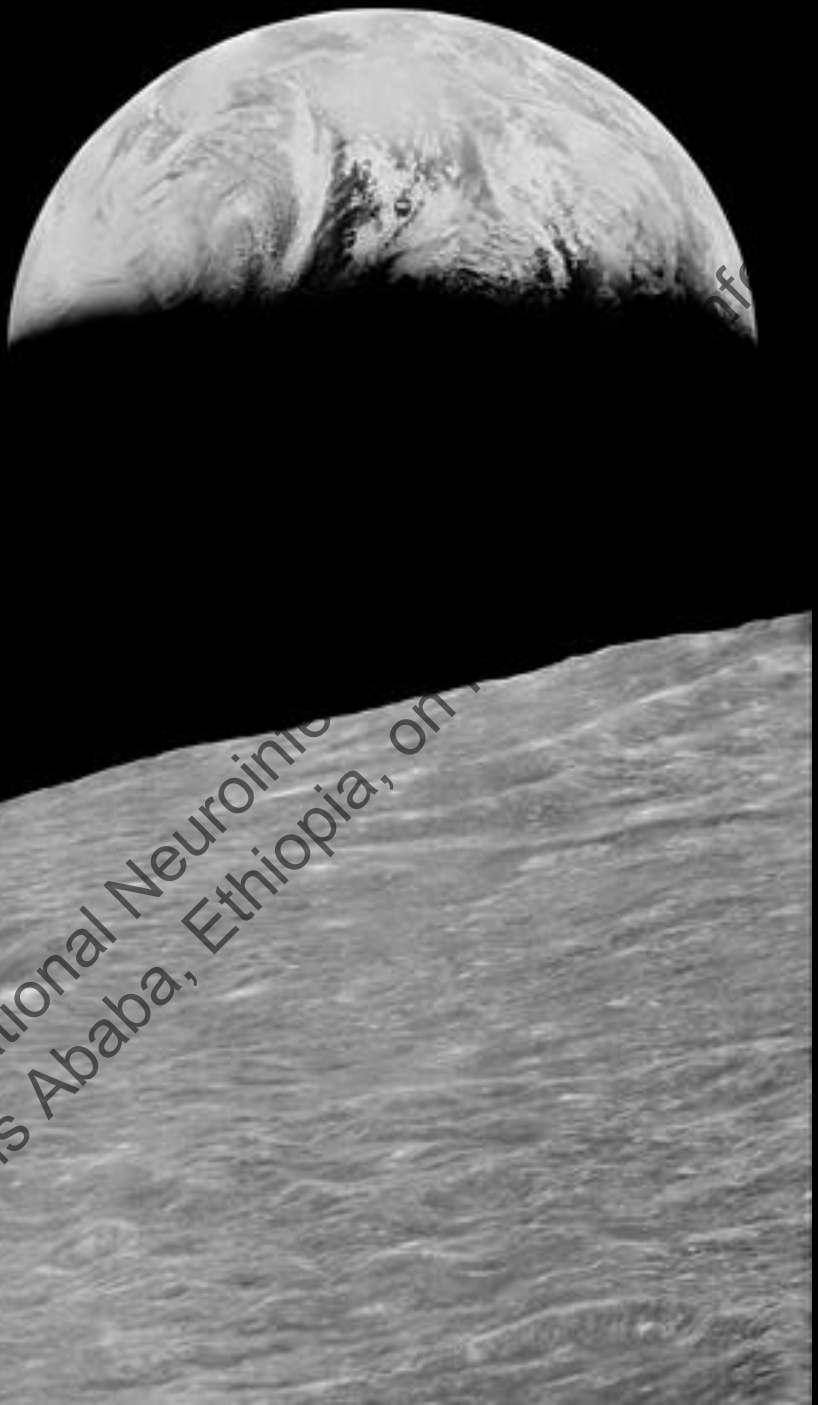
*200ul sample concentrated to 25ul; 10ul used for assay in duplicate; 40 cycles only

*DNA for standards i.e. viral DNA isolated/purified from infected cell cultures. Control sample tests from plasmid or other source of viral DNA

Next Steps

- Nature of standards
- JCV ‘ccc’ DNA from Hirt extracts
- Concensus on template extraction
- Report on the data from proficiency tests in 2007

- Publish a ‘Standards’ paper i.e. Berlin, Milan meetings
  - EU organization; Society for Genome Amplification Technology
  - USA organization; College of American Pathology (CAP)
  - Suggestion to Journals to ask authors about certification/validation of assays



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