510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K112394

B. Purpose for Submission:

New Device

C. Measurand:

Anti- John Cunningham Virus (JCV) antibodies

D. Type of Test:

Enzyme Linked Immunosorbent Assay (ELISA)

E. Applicant:

Focus Diagnostics Inc.

F. Proprietary and Established Names:

STRATIFY JCV™ Antibody ELISA

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3336: John Cunningham Virus serological reagents

2. Classification:

   Class II (de novo)

3. Product code:

   OYP

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

The STRATIFY JCV™ Antibody ELISA testing service provided by Focus Diagnostics is intended for the qualitative detection of antibodies to John Cunningham virus in human serum or plasma. The assay is intended for use in conjunction with other clinical data, in multiple sclerosis and Crohn's disease patients receiving natalizumab therapy, as an aid in risk stratification for progressive multifocal leukoencephalopathy development. The assay is for professional use only and is to be performed only at Focus Diagnostics' Reference Laboratory.

The assay is not intended for donor screening. The performance of this assay has not been established for use in other immunocompromised patient populations or in neonates and pediatrics patient populations.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

N/A

4. Special instrument requirements:

N/A

I. Device Description:

The STRATIFY JCV™ Antibody ELISA consists of two devices. The first is an initial detection ELISA and the second device is a confirmatory (inhibition) ELISA. Both tests utilize the same recombinant antigen which is used in two different formats as described in section L. The devices include the following reagents; Recombinant JC virus like particles (VLP), a JCV high and low positive controls, consisting of human sera that is positive for JCV antibodies, and JCV negative control consisting of human sera that is negative for JCV antibodies. The conjugate, substrate, wash buffers and blocking buffers needed for the test are not supplied with the device and are listed together with other reagents and consumables in the device labeling.

J. Substantial Equivalence Information:

1. Predicate device name(s):

None

2. Predicate 510(k) number(s):
   None

3. Comparison with predicate:
   N/A

K. Standard/Guidance Document Referenced (if applicable):

A Special Controls Guidance Document will be promulgated.

L. Test Principle:

The STRATIFY JCV™ Antibody ELISA, consists of two assays performed in 2 steps. The first test detects anti-JCV antibodies and the second test is a confirmation (inhibition) assay to confirm the indeterminate results obtained by the first assay. In the first step (or assay) JCV-VLP are coated onto 96-well microtiter plates. Samples and controls are incubated in the wells to allow the binding of JCV specific antibodies in the samples to the JCV-VLP in the solid phase. After washing away the unbound reactants the plates are incubated with horseradish peroxidase labeled donkey anti-human IgG (H+L). Then, the excess conjugate is washed and the reaction developed using tetramethylbenzidine peroxidase substrate and stopped by the addition of a diluted acid stop solution. The results are read as optical density (OD) values by spectrophotometry. Results are recorded as a normalized value (nOD). Samples with nOD values greater than 0.25 are reported as positive for detectable anti-JCV antibodies, samples with nOD values less than 0.1 are reported as negative for detectable anti-JCV antibodies. Samples with nOD values between 0.25 and 0.1 are reported as indeterminate and requiring evaluation in the confirmation (inhibition) assay.

The second step is intended to identify samples containing antibodies which cross react with denatured capture antigen or with other polyomaviruses such as BK virus (BKV). In the second step, the confirmation (inhibition) assay, samples are pre-incubated with the JCV-VLP in solution prior to the incubation in the JCV-VLP-coated plates, competing with the plate bound JCV-VLP. JCV specific antibodies will bind to the free VLP. The ELISA is then completed as described for the first step. False positive results are characterized by the minimum competition. The percent inhibition is calculated to confirm the presence of anti-JCV antibodies. Samples with a percent inhibition > 40% are reported as confirmed positive, and samples with percent inhibition ≤ 40% are reported as confirmed negative.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   The precision of the detection assay was characterized in one laboratory. The

study design included testing a panel of serum and plasma samples by three operators, two runs per day over a period of 5 non-consecutive days spread across two weeks. Each sample pool was diluted in three aliquots; each individual aliquot was assayed in duplicate providing one nOD result per aliquot. The STRATIFY JCV™ Antibody ELISA detection assay demonstrates a total % CV ranging from 7.0% to 18.3% for the plasma pools and 9.1% to 12.9% for the serum pools. The serum indeterminate pool returned an indeterminate result 96.7% (87/90) of the time, the plasma indeterminate pool returned an indeterminate result 100% of the time. The confirmation (inhibition) assay was performed in batches by random operators on the indeterminate pools. The indeterminate pool was confirmed as positive in the inhibition assay 100% (86/86) of the time for the serum indeterminate pool and 100% (90/90) for the plasma indeterminate pool.

Precision - Detection Assay (nOD values)

Sample Matrix	Sample Level	*Qualitative Result using nOD			N	Mean	Precision Component									
							Between Operators		Between Days		Between Runs		Within-Run (repeatability)		Total	
		D	I	ND			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Plasma	High Positive	90			90	0.993	0.036	3.605	0.029	2.960	0.041	4.083	0.032	3.178	0.069	6.97
	Indeterminate		90		90	0.150	0.003	2.045	0.011	7.402	0.006	4.010	0.006	3.953	0.014	9.52
	Low Positive	87	3		90	0.337	0.038	11.29	0.037	10.82	0.027	7.861	0.017	5.172	0.062	18.25
	Negative		6	84	90	0.082	0.001	0.775	0.009	10.79	0.006	6.842	0.003	3.811	0.011	13.36
Serum	High Positive	90			90	0.978	0.061	6.218	0.091	9.253	0.039	3.994	0.046	4.712	0.125	12.75
	Indeterminate		87	3	90	0.116	0.000	0.000	0.008	6.570	0.004	3.518	0.006	5.136	0.011	9.05
	Low Positive	86	4		90	0.296	0.000	0.000	0.033	11.06	0.013	4.346	0.014	4.876	0.038	12.85
	Negative			90	90	0.058	0.000	0.000	0.004	6.586	0.003	4.513	0.003	4.616	0.005	9.22

* D = Detected, I = Indeterminate, ND = Not Detected

Precision - Confirmation Assay

Sample	*Qualitative Result using % Inhibition		N	Descriptive Statistics of % Inhibition						
	D	ND		Min	5th Percentile	95th Percentile	Max	Mean	SD	%CV
Indeterminate (Plasma)	90		90	61.11	62.93	80.10	81.50	71.57	4.97	6.95
Indeterminate (Serum)	86**		86	44.71	47.40	65.71	68.54	55.28	5.26	9.52

* D = Detected, ND = Not Detected

** Three replicates of this sample were negative in the detection assay, per the laboratory procedure the confirmation assay is not performed on negative samples. One dilution was inadvertently omitted by the operator.

b. Linearity/assay reportable range:

N/A

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

N/A

d. Detection limit:

N/A

e. Analytical specificity:

Cross Reactivity

Cross reactivity was evaluated in a two part study. The first part of the study evaluated cross reactivity with commercially available human antibodies spiked into JCV negative serum and plasma as determined by the STRATIFY JCV™ Antibody ELISA. The potentially cross reacting antibodies were spiked at high concentrations. The concentrations tested and the test results are presented in the table below. Each antibody tested was not detected with the STRATIFY JCV™ Antibody ELISA. There was no observed reactivity with the three potentially cross-reacting antibodies tested.

Cross Reactivity - Part One – Spiked Antibodies

Cross Reactant	Concentration	Quantity Detected

		Serum	Plasma
Antibody to *Escherichia coli*	0.4 µg/mL	0/3	0/3
Antibody to *Mycobacterium tuberculosis*	0.4 µg/mL	0/3	0/3
Antibody to *Pneumocystis jiroveci*	unquantified	0/3	0/3

In the second part of the study a panel of up to twenty remnant specimens that previously tested positive for each of the potential cross reacting antibodies was evaluated. Each member of the panel was evaluated using the STRATIFY JCV™ Antibody ELISA along with appropriate controls. The seroprevalence of JCV for each group of potential cross reactants in the panel was compared to the expected seroprevalence of JCV in the normal population. If a group demonstrated a higher than expected seroprevalence of JCV it was considered as an indicator of potential cross reactivity.

Three groups of patients (*C. pneumoniae*, HSV1 and *T. pallidum*) exhibited a positivity rate that was slightly above that observed in previously reported studies; one group of patients (*M. pneumoniae*) exhibited a positivity rate that was lower than the observed rate in previous studies. The results suggest that these groups demonstrate potential cross reactivity.

Cross Reactivity – Part Two – Sero-prevalence Comparison

Sero-positive Samples	Antibodies to JCV Detected	Quantity Tested	% Positive
C. pneumoniae	15	20	75.0%
C. trachomatis	11	20	55.0%
HSV 6	11	20	55.0%
Varicellea Zoster Virus	12	20	60.0%
Candida	13	20	65.0%
HSV 1	17	20	85.0%
HSV 2	7	20	35.0%
Treponema pallidum	4	4	100.0%
Mycoplasma pneumoniae	1	9	11.1%
CMV	10	15	66.7%
HIV 1	14	20	70.0%
Overall	**115**	**188**	**61.2%**

Interferences

A two-fold approach was used to evaluate potential interference due to endogenous substances and concomitant medications. Endogenous substances were studied in two studies as described in the following paragraphs. The potential interference caused by concomitant medications was evaluated by reviewing clinical information provided as part of the drug trials. Potential interference due to endogenous substances were evaluated using a sample panel of native sera and plasma samples that contain a low concentration of

JCV antibody which are spiked with the potential interferent as compared to baseline of the same serum and plasma samples that did not contain the interferent. The interferents were spiked at the highest possible endogenous level. For all of the potential interferents with the exception of γ globulin; the observed differences in signal did not cause any changes in interpretation of the final result. Plasma spiked with triglyceride, hemogolobin, and bilirubin and sera spiked with hemoglobin and bilirubin demonstrated a >20% shift from baseline, indicating potential interference. Commercially available γ globulin is produced using normal human serum containing IgG antibodies; since the seroprevalence of antibodies to JCV virus is approximately 55% in the normal population γ globulin is expected to react with this assay.

Interference Summary – Signal Comparison to Baseline

Potential Interferent Panel	Plasma			Serum		
	Mean nOD	Std Dev	%Change from Baseline	Mean nOD	Std Dev	%Change from Baseline
Baseline (Part 1)	0.385	0.031	N/A	0.440	0.025	N/A
Triglyceride (10 mg/mL)	0.295	0.003	-23.3%	0.411	0.017	-6.4%
Hemoglobin (200 mg/mL)	0.283	0.008	-26.3%	0.348	0.013	-20.9%
Bilirubin (0.2 mg/mL)	0.289	0.008	-24.9%	0.337	0.005	-23.3%
Baseline (Part 2)	0.315	0.0074	N/A	0.296	0.0139	N/A
Albumin (12 g/dL)	0.276	0.0083	-12.6	0.267	0.0172	-9.6
Ascorbic Acid (3 mg/dL)	0.284	0.0451	-9.9	0.273	0.0199	-7.9
Cholesterol (500 mg/dL)	0.302	0.0175	-4.4	0.289	0.0120	-2.2
γ globulin (6 g/dL)	1.206	0.0136	282.4	1.308	0.0616	342.1

[1.] The observed decrease in signal may be in part due to a decrease in JCV antibody concentration caused by the additional volume of the spiked interferent

Interference Due To Concomitant Medications

In order to evaluate the potential effect of concomitant medications on the ability to detect JCV antibodies, results were evaluated for serum samples collected serially over a period of 30 months from a subset of 585 MS patients in the AFFIRM study using the STRATIFY JCV™ Antibody ELISA. AFFIRM is a completed clinical trial where patients with multiple sclerosis were randomized to receive placebo or natalizumab, and blood samples were collected every 6 months for 30 months. The AFFIRM study utilized an

ongoing Concomitant Medications Log for each patient to capture all concomitant medications used by the patient during the course of the study. A total of 557 of the 585 patient subset (95%) used concomitant medications during the 30 month AFFIRM study

In order to assess whether the presence of concomitant medications caused interference in the STRATIFY JCV™ Antibody ELISA, each of the concomitant medications used by ≥10% and ≥20% of the patients in AFFIRM study were evaluated separately. The proportion of patients who tested: JCV antibody positive at any time point, JCV antibody positive at all time points, or JCV antibody negative at all time points over the 30 month study period were evaluated in those patients who received or did not receive each individual concomitant medication. If interference occurred at any time point over the 30 month sampling period due to a particular concomitant medication, the proportion of patients who tested JCV antibody positive or negative in these categories would be expected to be different in those who received the medication compared to those patients who did not. The following table illustrates the results of the study.

Concomitant Medications Used AFFIRM Who Also Provided Longitudinal Samples Tested for JCV Antibody Status

MEDICATION	Total Number of Patients Who Received a Concomitant Medication (%)*	JCV ANTIBODY STATUS*	PATIENTS REPORTING MEDICATION USE		PATIENTS NOT REPORTING MEDICATION USE	
			Sample Size	Percent	Sample Size	Percent
Paracetamol	238 (41%)	Positive at ANY time point	151	63 (57.0to69.6)	209	60 (54.9to65.4)
		Positive at ALL time points	122	51 (44.7 to 57.8)	167	48 (42.8 to 53.5)
		Negative at ALL time points	87	37 (30.4-43.0)	138	40 (34.6 to 45.1)
Ibuprofen	211 (36%)	Positive at ANY time point	125	59 (52.3 to 65.9)	235	63 (57.7 to 67.7)
		Positive at ALL time points	96	45 (38.6 to 52.5)	193	52 (46.4 to 56.8)
		Negative at ALL time points	86	41 (34.1 to 47.7)	139	37 (32.3 to 42.3)

MEDICATION	Total Number of Patients Who Received a Concomitant Medication (%)*	JCV ANTIBODY STATUS*	PATIENTS REPORTING MEDICATION USE		PATIENTS NOT REPORTING MEDICATION USE	
			Sample Size	Percent	Sample Size	Percent
Methylprednisolone	167 (29%)	Positive at ANY time point	107	64 (56.3 to 71.3)	253	61 (55.7 to 65.2)
		Positive at ALL time points	91	54 (46.6 to 62.2)	198	47 (42.5 to 52.3)
		Negative at ALL time points	60	36 (28.7 to 43.7)	165	39 (34.8 to 44.3)
Amoxicillin	115 (20%)	Positive at ANY time point	71	62 (52.2 to 70.6)	289	61 (56.9 to 65.9)
		Positive at ALL time points	53	46 (36.8 to 55.6)	236	50 (45.6 to 54.8)
		Negative at ALL time points	44	38 (29.4 to 47.8)	181	39 (34.1 to 43.1)
Acetylsalicylic Acid	78 (13%)	Positive at ANY time point	51	65 (53.8 to 75.8)	309	61 (56.5 to 65.2)
		Positive at ALL time points	45	58 (46.0 to 68.8)	244	48 (43.7 to 52.6)
		Negative at ALL time points	27	35 (24.2 to 46.2)	198	39 (34.8 to 43.5)
Multi -Vitamin	69 (12%)	Positive at ANY time point	39	57 (44.0 to 68.4)	321	62 (57.9 to 66.4)
		Positive at ALL time points	27	39 (27.6 to 51.6)	262	51 (46.4 to 55.2)
		Negative at ALL time points	30	43 (31.6 to 56.0)	195	38 (33.6 to 42.1)
Amantadine	73 (12%)	Positive at ANY time point	46	63 (50.9 to 74.0)	314	61 (57.0 to 65.6)

MEDICATION	Total Number of Patients Who Received a Concomitant Medication (%)*	JCV ANTIBODY STATUS*	PATIENTS REPORTING MEDICATION USE		PATIENTS NOT REPORTING MEDICATION USE	
			Sample Size	Percent	Sample Size	Percent
		Positive at ALL time points	36	49 (37.4 to 61.3)	253	49 (45.0 to 53.8)
		Negative at ALL time points	27	37 (26.0 to 49.1)	198	39 (34.4 to 43.0)
Diclofenac	66 (11%)	Positive at ANY time point	34	52 (38.9 to 64.0)	326	63 (58.5 to 67.0)
		Positive at ALL time points	26	39 (27.6 to 52.2)	263	51 (46.3 to 55.1)
		Negative at ALL time points	32	48 (36.0 to 61.1)	193	37 (33.0 to 41.5)

The proportion of patients in each category was consistent between those patients who received the medications and those who did not. The results did not show any potential interfering effect of these commonly used medications on the performance of the assay.

f. Assay cut-off:

To characterize antibody responses against infectious agents in humans, it is critical to have reference sera from both infected and non-infected individuals However, JCV infection is clinically asymptomatic, thereby making it difficult to generally distinguish JCV infected from non-infected individuals. Therefore it is difficult to obtain samples from confirmed infected and non-infected individuals. Even though about 20-30% of JCV infected individuals shed viral DNA in the urine, JCV DNA is often not detected in the blood or urine of infected individuals, even when the infection results in the development of PML. There is no evidence that testing for JCV DNA in blood or urine can identify all JCV infected individuals, however, those who shed virus in the urine are confirmed to be infected with JCV. Therefore, sera from viruric patients were used to establish the positive reference sera for the ELISA. The assay cut point was established from the distribution of the serological responses of samples collected from JCV viruric patients in the JCV antibody ELISA. None of the JC viruric subjects had a reactivity below 0.1, which was therefore selected as the lower cut point in the JCV antibody

ELISA.

The false negative rate of the assay was calculated from the number of subjects who were urinary JCV DNA positive (viruric), but negative for serum JCV antibodies in the STRATIFY JCV™ Antibody ELISA. An initial assessment of the STRATIFY JCV antibody ELISA false negative rate of 2.5% (95%CI: 0.5 to 4.9%) resulted from 5 of 204 viruric patients from the STRATA study whom tested seronegative. The false negative rate of the JCV antibody assay was confirmed using serum and urine samples collected at enrollment from 1073 subjects in the STRATIFY-1 study. Of the 1073 subjects, 186 (17%) were found to be urinary JCV DNA positive. The false negative rate of the assay in STRATIFY-1 was determined to be 2.7% (95%CI: 0.9 to 6.2%) as 5 out of the 186 urinary JCV DNA positive subjects did not have detectable serum JCV antibodies. The seropositivity rate for CD patients was assessed using the same cut-off points and found to be similar to that of MS patients. No matrix differences were found between MS patients, CD patients or healthy volunteers. The false negative rate for CD population was not determined; however, the data supports the applicability of the cut-off and the false negative rate established for MS population to the CD population.

2. Comparison studies:

a. Method comparison with predicate device:

There is no predicate device for this assay. However, the sponsor performed a method comparison study with a laboratory developed test for the detection of anti-JCV antibodies using a panel of 100 samples that cover the detection range of their assay. The purpose of this study was to evaluated the comparative performance with a different anti-JCV antibody detection test particularly in samples close to the cut-off of the STRATIFY Antibody ELISA. The sample panel consisted of 62 negative, 71 low positives near the cut-point and 67 positive samples. Low positives include samples between two cut points (0.1 and 0.25) and a small group of samples just above the upper cut point (the range is from nOD ≤ 0.1 to ≤0.35). The Positive percent agreement (PPA) was 91.0% (122/134) with 95% CI: 85.0% to 94.8%, the Negative percent agreement (NPA) was 89.4% (59/66) with 95% CI: 79.7% to 94.8%. The results of the analytical comparison are shown below.

		Comparative Assay					
		Detected			**Not Detected**		
		Sample Category based on initial nOD			Sample Category based on initial nOD		
		Positive (nOD>0.35)	Low Positive* (0.1 ≤ nOD ≤ 0.35)	Negative (nOD < 0.1)	Positive (nOD> 0.35)	Low Positive* (0.1 ≤ nOD ≤ 0.35)	Negative (nOD < 0.1)

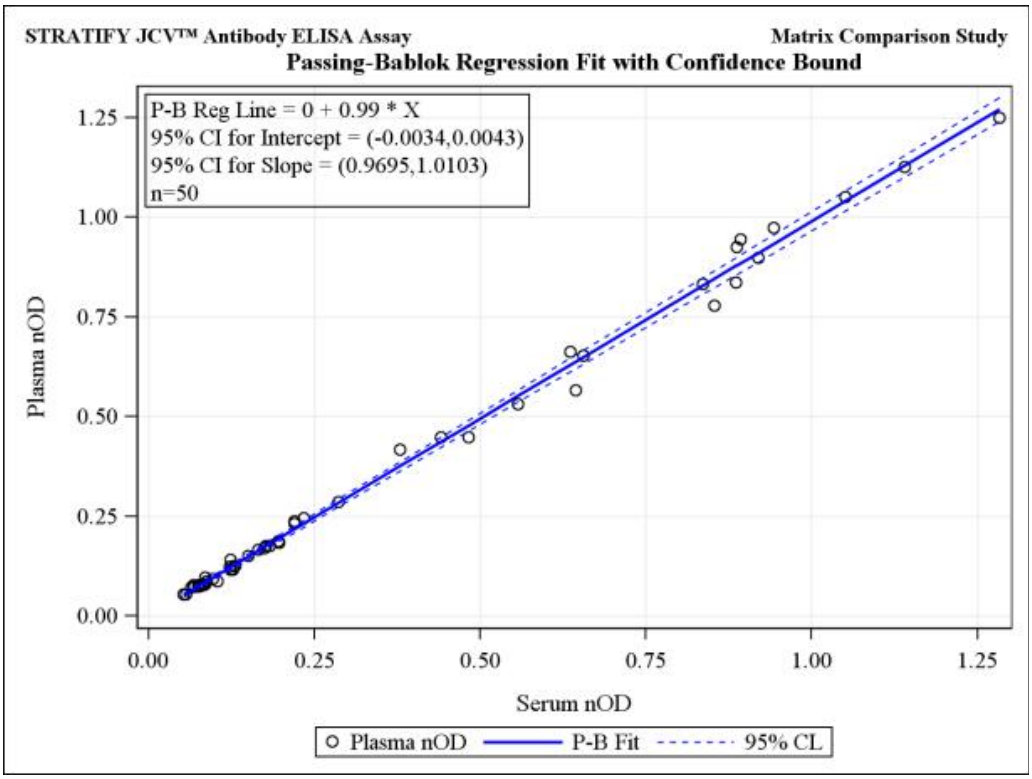
STRATIFY JCV Antibody ELISA test	**Positive**	67	55	0	0	7**	0
	Negative	0	2**	10	0	7**	52
	Total	67	57	10	0	14	52
		134			66		
		200					
* 63 out of 71 samples fell in screening "Indeterminate" zone ((0.1 ≤ nOD ≤ 0.25) which were followed by confirmation (%Inhibition) in Focus assay. **2+7+7=16 samples were part of 63 samples which required confirmation assay in Focus assay.							

b. Matrix comparison:

A total of fifty paired plasma and serum samples were assayed to evaluate the equivalence between both sample types. The comparison of the qualitative results from the serum and plasma samples is presented in the table below. A graphical representation of the scatter plot of the data and the Passing-Bablok regression analysis of nOD values are shown in the following figure. The slope was 0.99 with 95% CI: 0.9695 to 1.0103 and an intercept was 0.0 with 95% CI: -0.0034 to 0.0043. The mean % difference for nOD values obtained for serum and plasma samples was 4.9%. Additionally, 50 out of 50 (100%) qualitative results obtained for plasma samples matched the qualitative results obtained for matched serum samples. The results demonstrate the equivalency of both matrices when used on the STRATIFY JCV™ Antibody ELISA.

Sample Matrix Comparison – Qualitative Results

Result based on Detection Assay nOD				
Plasma	Serum			
	Not Detected	Indeterminate	Detected	**Total**
Not Detected	15	1		**16**
Indeterminate		16		**16**
Detected			18	**18**
Total	**15**	**17**	**18**	**50**



3. Clinical studies:

   a. Clinical Sensitivity:

   N/A

   b. Clinical specificity:

   N/A

   c. Other clinical supportive data (when a. and b. are not applicable):

Because PML is an infrequent event in natalizumab-treated patients, data collected from both clinical trial and post-marketing reports of confirmed cases of PML were used to assess the clinical performance of the STRATIFY JCV™ Antibody ELISA test for PML risk stratification. A clinical plan was developed for collection of serum samples obtained from natalizumab-treated patients prior to the onset of PML for JCV antibody testing. As of September 2011, 37 available serum samples from confirmed PML patients collected at least 6 months prior to clinical diagnosis of PML were tested for JCV antibody status using the STRATIFY JCV™ Antibody ELISA. A total of 36 of these patients had received at least 18 infusions of natalizumab at the time of PML

diagnosis, while one patient had received 17 infusions of natalizumab. All 37 pre-PML samples tested positive for JCV antibodies with an estimated sensitivity of 100% (37/37) with 95% CI: 90.6% to 100%. In addition, there has only been one natalizumab treated CD patient with an available pre-PML sample who has developed PML. This sample also tested positive for JCV antibodies.

Samples from 5,896 MS patients were tested by the STRATIFY JCV Antibody ELISA. A total of 3,239 patients tested positive for antibodies to JCV using the test, and the positivity rate was estimated to be 54.9% (3,239/5,896) with 95% CI: 53.7% to 56.2%. An assay is statistically informative if the percentage of positive results in patients with the disease of interest is higher than the percentage of positive results in the population at risk. The 100% JCV antibody positivity demonstrated in the 37 natalizumab-treated PML patients prior to PML diagnosis was significantly different than the 54.9% JCV antibody positivity in the MS population, and represents a 1.82-fold (95% CI: 1.65 to 1.86) increased risk of PML compared to the PML incidence in the overall natalizumab-treated population.

Estimated incidence of PML by STRATIFY JCV Antibody ELISA test results based on statistical modeling with an assumption that there is one hypothetical PML case with a negative test result (38 PML cases: 37 positive and 1 hypothetical negative) and assumption that study has 13,227 patients and all they were tested by STRATIFY JCV Antibody ELISA test.

		Number with PML	Number without PML	Total number patients treated
STRATIFY JCV Antibody ELISA test	Positive	37	7,229	7,266
	Negative	1*	5,960	5,961
Total		38	13,189	13,227
Risk of PML (per 1,000) treated with ≥ 18 months for Positive result		5.09 95% CI: 3.70 to 7.01		
Risk of PML (per 1,000) treated with ≥ 18 months for Negative result		0.17 95% CI: 0.03 to 0.95		
Relative risk		30.4 95% CI: 5.3 to 437.4		

*For the negative result, a hypothetical case was assumed in order to allow for calculation of the point estimate.

Data from three studies performed in MS patients were analyzed to determine whether use of prior immunosuppressant (IS) therapy affects JCV antibody status. The data showed that the percentage of positive results obtained by the STRATIFY JCV Antibody ELISA test was not dependent on prior IS therapy. Data from the STRATIFY-1 study

was analyzed to determine whether duration of natalizumab treatment affects JCV antibody status. The data showed that the percentage of positive results obtained by the STRATIFY JCV Antibody ELISA test analyzed by dosing interval of natalizumab was not dependent on treatment duration.

The STRATIFY JCV antibody ELISA testing service results can therefore be used along with the other established PML risk factors of prior immunosuppressant use and natalizumab treatment duration, to stratify an individual patient's risk of PML. JCV antibody positive status results in a 1.82-fold increased risk of PML. The risk of PML based on prior IS use and treatment duration is therefore multiplied by 1.82 to estimate the PML risk based on the three risk factors combined

Risk of PML in JCV Antibody Positive Patients Based on Prior IS Use and Treatment Duration (per 1,000)

Natalizumab Exposure†	STRATIFY JCV Antibody ELISA test Positive Result*	
	No Prior Immunosuppressant Use	Prior Immunosuppressant Use
1-24 months	<1/1,000	2/1,000
25-48 months	4/1,000	11/1,000

Notes: Based on postmarketing PML data and natalizumab use data as of September 1, 2011.
†Data beyond 4 years of treatment are limited.
*Risk in anti-JCV antibody positive patients was estimated based on the assumptions that 18% of Natalizumab-treated MS patients have a history of prior immunosuppressant treatment and that 55% of natalizumab-treated MS patients are anti-JCV antibody positive.

4. Clinical cut-off**:**

   N/A

5. Expected values/Reference range:

   The STRATIFY JCV™ Antibody ELISA has been used to evaluate JCV antibody positivity rate in serum and plasma samples from a geographically diverse cohort of 5,896 MS patients. The sample cohort was comprised from MS patients from clinical trials including a completed Phase 3 clinical study of natalizumab in MS (AFFIRM C-1801), an ongoing study to evaluate seroprevalence in the MS population (STRATIFY-1 [101JC401]), an observational study in natalizumab treated MS patients (TYGRIS-US [101MS402]) and a national MS registry from Sweden. The clinical characteristics for the MS patients within each study are shown in Table 2. The age and gender distribution of the MS cohort tested with

the STRATIFY JCV™ Antibody ELISA are similar to the age and gender distribution of MS patients treated with natalizumab in the post-marketing setting. JCV antibody positivity rate in the MS cohort was 47-59% which is consistent with what has been reported in the literature. JCV antibody prevalence was shown to increase with age and was lower in women compared to men which is also consistent with what has been reported in the literature in healthy adults using similar assay methodologies

Demographic Data and JCV Antibody Prevalence for MS Patients

	AFFIRM (N=823)	**TYGRIS-US (N=1480)**	**STRATIFY-1 (N=1096)**	**Swedish MS Registry (N=2497)**
Age (years)				
• Range	18-50	18-75	12-75	12-75
• Mean	35.9	44.3	44.4	37.5
• Median	36	44	45	37
Gender (%)*				
• Male	252/823 (30.6%)	350/1451 (24.1%)	266/1096 (24.3%)	700/2494 (28.1%)
• Female	571/823 (69.4%)	1101/1451 (75.9%)	830/1096 (75.7%)	1794/2494 (71.9%)
Geography	North America and EU/Rest of World	US and Canada	US	Sweden
JCV Antibody Positivity Rate (95% CI)				
• % JCV Antibody Positive	449/823 (54.6%) (51.1 to 58.0)	704/1480 (47.6%) (45.0 to 50.1)	614/1096 (56.0%) (53.0 to 59.0)	1472/2497 (59.0%) (57.0 to 60.9)
• % JCV Antibody Negative	374/823 (45.4%) (42.0 to 48.9)	776/1480 (52.4%) (49.9 to 55.0)	482/1096 (44.0%) (41.0 to 47.0)	1025/2497 (41.0%) (39.1 to 43.0)

* In the TYGRIS-US dataset, 1451 of the 1480 patients had age and gender information available. In the Swedish MS dataset, 2464 of 2497 patients had age information available and 2494 of 2497 patients had gender information available.

The STRATIFY JCV™ Antibody ELISA has also been used to evaluate JCV antibody prevalence in serum samples from a geographically diverse cohort in CD patients. The sample cohort was comprised of patients from two completed Phase

3 clinical trials of natalizumab in CD (CD301 and CD303). The clinical characteristics for the CD patients within these studies are shown in Table 3. The age and gender distribution of the CD cohort tested with the STRATIFY JCV™ Antibody ELISA are similar to the age and gender distribution of CD patients treated with natalizumab in the post-marketing setting. JCV antibody prevalence in the CD cohort was 55.6% and was shown to increase with age, consistent with what has been reported in the literature 9, 12. In general there was no difference in the prevalence of JCV antibodies between genders.

Demographic and JCV Antibody Positivity Rate for CD Patients

	Clinical Studies CD301 and CD303 (N=313)
Age (years)	
• Range	18-74
• Mean	38.4
• Median	37
Gender (%)	
• Male	123/313 (39.3%)
• Female	190/313 (60.7%)
Geography	North America and EU/Rest of World
JCV Antibody Positivity Rate (95% CI)	
• % JCV Antibody Positive	174/313 (55.6%) (49.9 to 61.2)
• % JCV Antibody Negative	139/313 (44.4%) (38.8 to 50.1)

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.3336 with special controls. The special control guidance document "*Class II Special Controls Guidance Document: John Cunningham Virus Serological Reagents*," will be available shortly.