510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K120986

B. Purpose for Submission:

New device

C. Measurand:

Anti- JC Virus (JCV) antibodies

D. Type of Test:

Enzyme Linked Immunosorbent Assay (ELISA)

E. Applicant:

Focus Diagnostics Inc.

F. Proprietary and Established Names:

STRATIFY JCV® DxSelect™

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3336: John Cunningham Virus serological reagents

2. Classification:

   Class II Special Controls

3. Product code:

   OYP

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Focus Diagnostics' STRATIFY JCV® DxSelect™ assay is intended for the qualitative detection of antibodies to JC virus in human serum or plasma. The assay is intended for use in conjunction with other clinical data, in multiple sclerosis patients receiving or considering natalizumab therapy, as an aid in risk stratification for progressive multifocal leukoencephalopathy development. The assay is for professional use only.

   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 patients with different disease conditions or undergoing other treatments or in neonates and pediatric patient populations.

2. Indication(s) for use:

   The same as intended use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   None

I. Device Description:

The Focus Diagnostics' STRATIFY JCV® DxSelect™ 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):
   STRATIFY JCV™ Antibody ELISA

2. Predicate 510(k) number(s):
   K112394

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	The assay is intended for the qualitative detection of antibodies to JC virus in human serum or plasma.	Same.
Measurand	Anti-JCV antibodies	Same.
Technology	Detection ELISA with a secondary Confirmation ELISA for specimens that have results in the indeterminate zone levels.	Same.
Matrix (sample type)	Serum and Plasma	Same

Differences		
Item	Device	Predicate
Indications for use	The assay is intended for use in conjunction with other clinical data, in multiple sclerosis patients **receiving or considering natalizumab therapy**, as an aid in risk stratification for progressive multifocal leukoencephalopathy development. The assay is for professional use only. 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 pediatric patient populations.	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
Assay Cut-off points	Index < 0.2, negative	nOD < 0.1, negative
	Index ≥ 0.4, positive	nOD ≥ 0.2, positive

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

CLSI EP05-A2; Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline 2nd Edition (2004).

CLSI EP07-A2; Interference Testing in Clinical Chemistry; Approved Guideline 2nd Edition (2005).

CLSI H18-A4; Procedures for the Handling and Processing of Blood Specimens; Approved Guideline 4th Edition (2010).

L. Test Principle:

In the Focus Diagnostics' STRATIFY JCV® DxSelect™ test, JC virus-like particles (VLP) are pre-coated onto 96-well microtiter plates. Diluted serum or plasma specimens and controls are incubated in the wells to allow JCV-specific antibodies present in the specimens to react with the JC VLP antigen. Nonspecific reactants are removed by washing. Peroxidase-conjugated anti-human antibodies are added to react with JCV-specific antibodies. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD). Specimen OD readings are compared with Cut-Off Calibrator OD readings to determine results. Each specimen result is reported as an index value. A specimen with an index value that is greater than a specified upper cut-off is reported as positive for detectable JCV-specific antibodies, whereas a specimen with an index value less than the specified lower cut-off is reported as negative for detectable JCV-specific antibodies. A specimen with an index value that is equal to or between the upper and lower cut-off values is reported as indeterminate. An indeterminate result requires further evaluation in the confirmation (inhibition) assay.

In the confirmation assay, soluble JC VLP antigen will compete with plate bound JC VLP antigen for the JCV-specific antibodies present in the serum or plasma specimens. After washing away the unbound antibodies, peroxidase-conjugated anti-human antibodies are added and bind to any captured JCV-specific antibodies. Excess conjugate is removed by washing. Enzyme substrate and chromagen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of OD. The percent inhibition is calculated to confirm presence of JCV-specific antibodies in the specimen. Specimens with a percent inhibition value that is greater than the specified cut-off are reported as positive for detectable JCV-specific antibodies, whereas specimens with percent inhibition values less than or equal to the cut-off are reported as negative for detectable JCV-specific antibodies.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

Reproducibility

The reproducibility of the assay was assessed using a sample panel consisting of four levels of sera, four levels of (EDTA) plasma samples, low and high positive controls. Three replicates of each panel member were tested on two runs a day for a total of five days at two external testing sites and one internal site. The four levels included a negative, low, indeterminate and moderate positive samples prepared in a serum and a plasma matrices. The indeterminate samples were tested simultaneously in the confirmation assay and the detection assay.

Table: Reproducibility

JCV Gen-2 Validation Reproducibility Results																			
Parameter	**Sample Matrix**	**Sample Name**	**Qualitative Results**					**Quantitative Results**											
										Variability Components									
			ND	**I**	**D**	**NV**	**Total**	**N**	**Mean**	**Between Sites**		**Between Days**		**Between Runs/Operators**		**Within Assay (Repeatability)**		**Total**	
										SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
OD		Cut-Off						120	1.131	0.145	12.8	0.067	5.9	0.088	7.8	0.037	3.2	0.186	16.5
INDEX	Controls	Negative Control	89			1	90	89	0.102	0.010	9.5	0.007	7.1	0.010	9.7	0.006	6.1	0.017	16.5
		Positive Control			90		90	90	1.202	0.008	0.7	0.010	0.8	0.022	1.8	0.036	3.0	0.044	3.7
	Plasma	Indeterminate		90			90	90	0.270	0.010	3.7	0.009	3.4	0.011	4.2	0.014	5.1	0.023	8.3
		Low positive		39	51		90	90	0.410	0.000	0.0	0.000	0.0	0.022	5.5	0.023	5.7	0.032	7.9
		Medium positive			90		90	90	0.858	0.041	4.7	0.025	3.0	0.018	2.1	0.059	6.9	0.078	9.1
		Negative	89			1	90	89	0.129	0.002	1.8	0.007	5.5	0.013	9.8	0.008	5.8	0.017	12.8
	Serum	Indeterminate		90			90	90	0.283	0.012	4.3	0.000	0.0	0.011	3.9	0.018	6.3	0.024	8.5
		Low positive		18	72		90	90	0.422	0.004	0.9	0.004	1.1	0.018	4.2	0.013	3.1	0.023	5.4
		Medium positive			90		90	90	1.055	0.048	4.5	0.000	0.0	0.041	3.9	0.045	4.3	0.078	7.4
		Negative	84	2		4	90	86	0.109	0.024	22.3	0.000	0.0	0.018	16.5	0.013	11.7	0.033	30.1

JCV Gen-2 Validation Reproducibility Results																			
Parameter	Sample Matrix	Sample Name	Qualitative Results					Quantitative Results											
										Variability Components									
										Between Sites		Between Days		Between Runs/Operators		Within Assay (Repeatability)		Total	
			ND	I	D	NV	Total	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
%Inhibition	Plasma	Indeterminate	11		77	2	90	88	51.05	2.006	3.9	0.000	0.0	3.648	7.1	3.357	6.6	5.348	10.5
	Serum	Indeterminate	2		85	3	90	87	62.59	3.889	6.2	2.438	3.9	0.000	0.0	5.550	8.9	7.202	11.5

ND = Not Detected, I = Indeterminate, D = Detected, NV = Invalid

Reproducibility around the Assay cut-off

In order to demonstrate precision near the assay cut-off, two contrived samples, one serum and one (EDTA) plasma samples were prepared to be near the lower cut-point of the assay. Each sample was diluted twenty times and tested in the detection assay and the confirmation assay. As depicted in the following table the %CV was ≤ 2.6%.

Table: Reproducibility at the Limit of Detection

Sample Matrix	Descriptive Statistics of Detection Assay (Index)						Descriptive Statistics of Confirmation Assay (%Inhibition)					
	N	Min	Max	Mean	SD	%CV	N	Min	Max	Mean	SD	%CV
Plasma	20	0.33	0.36	0.34	0.01	2.3	20	61.66	66.97	65.11	1.43	2.2
Serum	19*	0.19	0.21	0.2	0.01	2.6	19	55.89	60.92	58	1.16	2

*One replicate was Invalid

b. Linearity/assay reportable range:

Not Applicable

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

Not Applicable

d. Detection limit:

Not Applicable

e. Analytical specificity:

Cross Reactivity

Cross reactivity was evaluated in a three part study conducted at an internal testing site.

I. The first study evaluated cross reactivity with commercially available human antibodies spiked into JCV negative serum and plasma as determined by the STRATIFY JCV® DxSelect™ assay. The potentially cross reacting antibodies were spiked in at concentrations estimated to be approximately 2-4 times higher than the estimated detection limit of JCV antibody. Each antibody tested was not detected with the STRATIFY JCV® DxSelect™ assay. There was no observed reactivity with the three potentially cross-reacting antibodies tested in this part of the study and the results are presented in the table below.

Table: Cross Reactivity - Part One – Spiked Antibodies

Cross Reactant	Concentration	Agents 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

II. The second part of the study included a panel consisting of a least twenty left over specimens that previously tested positive for each of the potential cross reacting antibodies. Each member of the panel was evaluated using the STRATIFY JCV® DxSelect™ assay along with appropriate controls. The seroprevalence of JCV in the panel was compared to the expected seroprevalence of JCV in the normal population. If a group demonstrates a higher than expected seroprevalence of JCV (set as >70% for the purposes of this study) it may be an indicator of potential cross reactivity. Four groups of patients (C. pneumoniae, HIV, CMV and HSV 1) exhibited a positivity rate that was slightly above that observed in previously reported studies. The results suggest that these groups demonstrate potential cross reactivity.

Table: Cross Reactivity – Part Two – Sero-prevalence Comparison

Sample Matrix	Cross Reactant	Total No. of Replicates or Samples	Screening Result Count (Based on Index)			Confirmation Result Count (Based on %Inhibition)			Final Interpretation			
									Count		%	
			D	IND	ND	Not Tested	D	ND	D	ND	D	ND
Unknown	HIV	22	16	2	4	20	1	1	17	5	77.3	22.7
Serum	C. pneumoniae	48	35	5	8	43	2	3	37	11	77.1	22.9
	C. trachomatis	20	11	2	7	18	1	1	12	8	60.0	40.0

	CMV	40	27	7	6	33	5	2	32	8	80.0	20.0
	Candida	40	26	6	8	34	1	5	27	13	67.5	32.5
	EBV	40	23	9	8	31	2	7	25	15	62.5	37.5
	HSV 1	48	32	6	10	42	2	4	34	14	70.8	29.2
	HSV 2	20	8	5	7	15	1	4	9	11	45.0	55.0
	HHV6	20	11	2	7	18		2	11	9	55.0	45.0
	Listeria	17	8	3	6	14		3	8	9	47.1	52.9
	Mycoplasma	20	10	4	6	16		4	10	10	50.0	50.0
	Treponema pallidum	19	12	2	5	17	1	1	13	6	68.4	31.6
	VZV	20	12	4	4	16	1	3	13	7	65.0	35.0
All		**334**	**208**	**48**	**78**	**286**	**15**	**33**	**223**	**111**	**66.8**	**33.2**

D= Detected, ND = Not Detected, IND = Indeterminate

III. The third part of the cross reactivity evaluation consisted of an evaluation of the potential cross reactivity with other polyoma viruses. This included a cross absorption study using BK virus like particles (VLP). A total of 40 clinical specimens that have previously tested positive for JCV antibodies in the STRATIFY JCV® DxSelect™ assay were tested in a confirmation style assay using JC VLP and BK VLP. A control set consisted of samples spiked with JC VLP at the same concentration of VLP that is used in the confirmation assay. The test samples were with spiked with BK VLP at the same concentration. The tests samples would be considered to be cross reactive if the % change in signal between the unspiked sample and the sample spiked with BK VLP is > 45%. The control set demonstrated % change in OD that was consistent with expectations. The test samples demonstrated % change in OD values due to BK VLP that ranged from -15 to 27%. No samples exhibited >45% change in the OD signal when spiked with BK VLP, indicating that the assay does not cross react with BKV.

The potential cross reactivity with other human polyoma viruses was not evaluated. However, additional analysis of the structure of the VP1 protein of other polyoma viruses indicates that BKV and JCV are more closely related (79.4% similarity) than other polyoma virus such as Merkel Cell virus and WU virus and KI virus (30.8 to 50.8 % similarity). Due to the differences in viral structure a similar experiment with other polyoma virus VLP was not conducted.

Interferences

Potential interference due to endogenous substances was evaluated using a testing panel consisting of sera and plasma samples that exhibit JCV antibody values close to the assay cut-off. The samples 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. A potentially interference is suspected if the % change from the baseline sample is > 20%. Commercially available γ globulin is produced using normal human serum containing IgG antibodies, due to the high seroprevalence of antibodies to JCV virus in the normal population this may explain the observed cross reactivity.

Table: Interference Summary – Signal Comparison to Baseline

Substance Name	Substance Concentration	Plasma			Serum		
		Average Index		%Change from Baseline	Average Index		%Change from Baseline
		Baseline Sample	Interference Sample		Baseline Sample	Interference Sample	
Albumin	120 mg/mL	0.35	0.38	8.6	0.43	0.37	-14.0
Ascorbic Acid	0.03 mg/mL	0.40	0.42	5.0	0.42	0.44	4.8
Bilirubin	0.2 mg/mL	0.33	0.33	0.0	0.42	0.38	-9.5
Cholesterol	5 mg/mL	0.48	0.40	-16.7	0.46	0.44	-4.3
Gamma Globulin	60 mg/mL	0.38	3.54	831.6	0.41	3.52	758.5
Hemoglobin	110 mg/mL	0.36	0.41	13.5	0.44	0.46	5.0
Hemoglobin	165 mg/mL		0.38	6.1		0.39	-10.1
Hemoglobin	220 mg/mL		0.35	-2.4		0.37	-16.0
Triglycerides	10 mg/mL	0.36	0.35	-2.8	0.40	0.37	-7.5

Note: Three dilutions of Gamma Globulin stock solution exhibited Index values - 3.22, 3.57 and 3.40.

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. 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 assay. The assay cut point was established from the distribution of the serological responses of samples collected from JCV viruric patients in the STRATIFY JCV® DxSelect™ assay. The lower cut point for the detection assay was set at an index of 0.2 along with an upper cut point at 0.4, and inhibition cut point of the confirmation assay was set at 45%. The assay has an estimated false negative rate of 2.2% based on a negative JCV antibody results for 4 out of 184 serum samples collected from viruric patients in the STRATIFY-1 trial.

2. Comparison studies:

a. Method comparison with predicate device:

See clinical studies

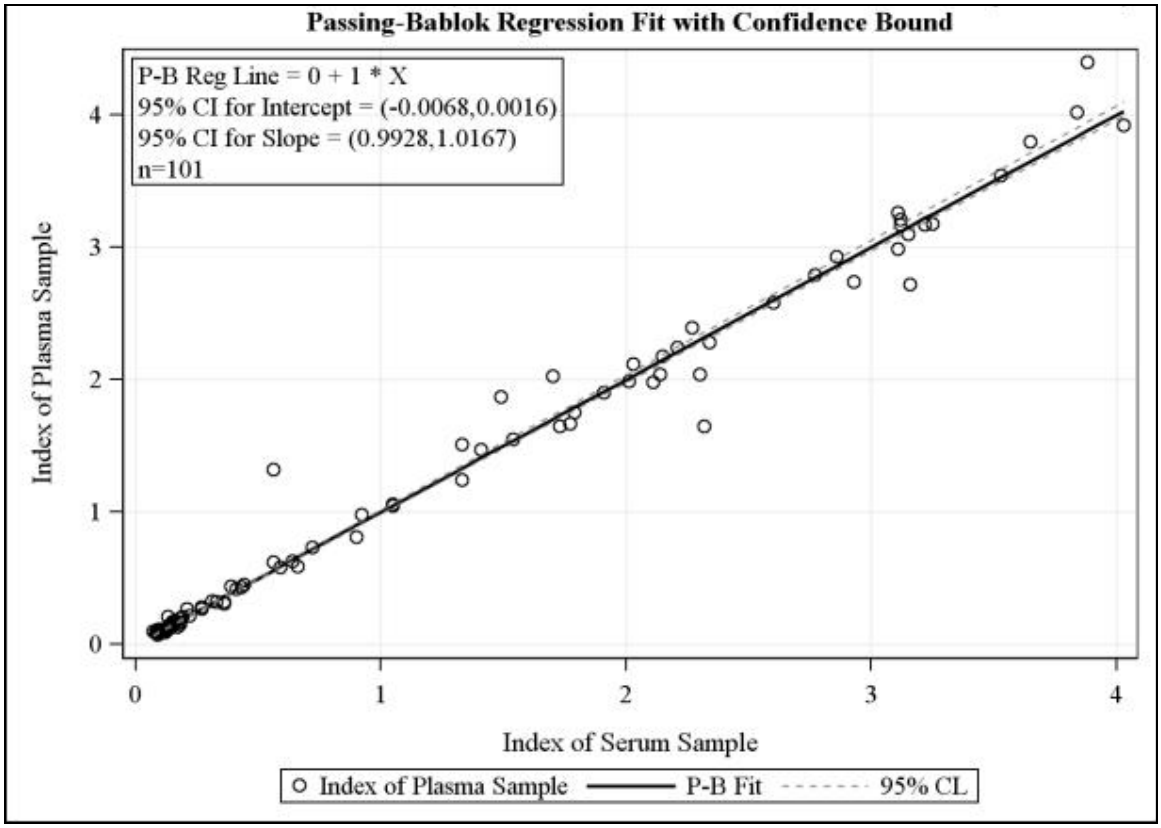
b. Matrix comparison:

A panel of 109 paired sera and (EDTA) plasma samples were evaluated in the STRATIFY JCV® DxSelect™ assay. Of the total 109 pairs one pair was eliminated from the regression analysis because the result of the sera sample was invalid, of the remaining 108 pairs 7 pairs were qualitatively detected with OD values that exceeded the range of the spectrophotometer. These pairs of samples are included in the qualitative result comparison below, but eliminated from the regression analysis since an index value could not be calculated. Passing-Bablok regression analysis of the pairs of sera and plasma specimens demonstrates a slope of 1 with a 95% CI of (0.9928 to 1.0167)) and an intercept of 0 with a 95% CI of (-0.0068 to 0.0016). Analysis of the qualitative results yielded Positive Percent Agreement = 96.7% (58/60), 95% CI: (88.6 to 99.1%), and Negative Percent Agreement = 97.9% (47/48), 95%CI: (89.1 to 99.6%)

Table: Sample Matrix Comparison - Sera vs. Plasma Qualitative Results

Final Plasma Result	**Final Serum Result (Count)**			**All**
	ND	**D**	**Invalid**	
ND	47	2	1	50
D	1	58		59
All	**48**	**60**	**1**	**109**

Figure: Passing-Bablok Regression Analysis (Sera vs. Plasma)



3. Clinical studies:

   a. Clinical Sensitivity:

   Not Applicable

   b. Clinical specificity:

   Not Applicable

   c. Other clinical supportive data (when a. and b. are not applicable):
   There is an increased risk of PML in natalizumab treated multiple sclerosis (MS) and Crohn's disease (CD) patients. Three risk factors for PML have been identified in MS and CD patient populations undergoing natalizumab treatment: natalizumab treatment duration, prior immunosuppressant use, and the presence of antibodies to JCV. Utilizing these three risk factors, sub-groups of patients can be identified with both higher and lower risk for PML. Please consult the current, locally available, prescribing or supplemental physician information for natalizumab for detailed information on the known risks associated with JCV serological status and the development of PML in natalizumab-treated patients. Patients with all three known risk factors have the highest risk for the development

of PML. The risk stratification for PML in natalizumab treated patients who are positive for the presence of JCV antibodies is shown in the following table.

Table: Estimated Incidence of PML Stratified by Risk Factor

Natalizumab Exposure†	JCV Antibody Positive *	
	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.

Clinical Performance (Comparative Agreement)

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® DxSelect™ assay 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. A total of 31 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® DxSelect™ assay. In addition to the pre-PML samples, 1330 Samples from MS patients were tested by the STRATIFY JCV® DxSelect™ assay. A total of 707 patients receiving treatment tested positive for antibodies to JCV using the test, and the positivity rate was estimated to be 58.7% (414/707) with a 95% CI of 54.9% to 62.1%. 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 31 natalizumab-treated PML patients prior to PML diagnosis was significantly different than the 58.7% JCV antibody positivity in the MS population, and represents an approximately 2-fold increased risk of PML compared to the PML incidence in the overall natalizumab-treated population.

In prior clinical studies for natalizumab the risk of developing PML was estimated using statistical modeling. The relative risk for patients who have received natalizumab for at least 18 months is shown in the table below. Risk was calculated 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 an assumption that the study has 13,227 patients.

Table: Estimated incidence of PML by JCV serological status

		Number with PML	Number without PML	Total number patients treated
JCV Serological Status	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.

The studies demonstrated that the positivity rate for JCV antibodies is not dependant upon prior IS use or the duration of natalizumab treatment. The results of the STRATIFY JCV® DxSelect™ assay can be used along with other established PML risk factors, of prior IS use and natalizumab treatment duration, to stratify an individuals risk for PML, please refer to Table 1 and to the prescribing information for additional risk estimates.

Performance with pre-PML samples.

The STRATIFY JCV® DxSelect™ assay was compared to an FDA cleared assay (STRATIFY JCV™ Antibody ELISA) using serum samples obtained from PML patients at least 6 months prior to PML diagnosis. 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® DxSelect™ assay for PML risk stratification**.** Thirty-one available serum samples from confirmed PML patients collected at least 6 months prior to clinical diagnosis of PML were tested at one internal testing site for JCV antibody status using the STRATIFY JCV® DxSelect™ assay and the validated laboratory methodology. The sample set demonstrated 100% (31/31) positive agreement (95% CI: 89.0% to 100%) with the validated assay.

Performance with archived clinical specimens

Two groups of prospectively collected and archived clinical samples obtained from the STRATIFY-2 and the AFFIRM clinical studies were used to assess the performance of the STRATIFY JCV® DxSelect™ assay compared to the validated laboratory methodology used in the STRATIFY-2 and AFFIRM clinical studies. One group consisted of patients who were receiving natalizumab, the other group of patients had not received natalizumab therapy (and were considering receiving natalizumab). The samples were blinded and randomly distributed to two external testing sites and one internal testing site. The data was analyzed for each group separately. Results for the two individual groups are presented in the tables below. The Positive and Negative Percent Agreement are shown in the table below.

Table: Agreement for Clinical Samples – Patients Receiving Natalizumab

STRATIFY JCV® DxSelect™	Predicate Device		Total
	Positive	Negative	
Positive	385	29	414
Negative	12	281	293
Total	397	310	707
Positive Percent Agreement (PPA)	97.0% (385/397) 95% CI: 94.8 to 98.3%		
Negative Percent Agreement (NPA)	90.6% (281/310) 95% CI: 86.9 to 93.4%		

Table: Agreement for Clinical Samples - Patients Considering Natalizumab

STRATIFY JCV® DxSelect™	Predicate device		Total
	Positive	Negative	
Positive	326	24	350
Negative	5	268	273
Total	331	292	623
Positive %Agreement (PPA)	98.5% (326/331) 95% CI: 96.5 to 99.4%		
Negative %Agreement (NPA)	91.8% (268/292) 95% CI: 88.1 to 94.4%		

4. Clinical cut-off**:**

   Not Applicable

5. Expected values/Reference range:

   The STRATIFY JCV® DxSelect™ assay been used to evaluate JCV antibody positivity rate in serum and plasma samples from a geographically diverse cohort of 1330 MS patients. The cohort was comprised from MS patients from clinical trials including a completed Phase 3 clinical study of natalizumab in MS patients (AFFIRM C-1801), an ongoing study to evaluate seroprevalence in the MS population (STRATIFY-2 [101JC402). The clinical characteristics for the MS patients within each study are shown in the following table. The age and gender distribution of the MS cohort tested with the STRATIFY JCV® DxSelect™ 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 55-59%. JCV antibody positivity rate was shown to increase with age and was lower in women compared to men, consistent with what has been reported in the literature in healthy adults using similar assay methodologies.

   Seroprevalence data was evaluated for each study. The observed seroprevalence using the STRATIFY JCV® DxSelect™ assay was 59% (467/792) in the STRATIFY-2 study and 55% (296/538) in the AFFIRM Study. These seroprevalence values are consistent with the seroprevalence values observed during the clinical studies, when the relationship between JCV serological status and PML development were described. Additionally the STRATIFY JCV® DxSelect™ assay demonstrated 100% concordance with 31 pre-PML samples.

Table: Demographic Data and JCV Antibody Prevalence for MS Patients

	AFFIRM (N=538)	**STRATIFY-2 (N=792)**
Age (years)		
• Range	18-50	19-78
• Mean	35.8	46.4
• Median	36	46
Gender (%)*		
• Male	173/538 (32.2%)	202/792 (25.5%)
• Female	365/538 (67.8%)	590/792 (74.5%)
Geography	North America and EU/Rest of World	US
JCV Antibody Positivity Rate (95% CI)		
• % JCV Antibody Positive	297/538 (55.2%) (51.0 to 59.4)	467/792 (59.0%) (55.5 to 62.3)
• % JCV Antibody Negative	241/538 (44.8%) (40.6 to 49.0)	325/792 (41.0%) (37.7 to 44.5)

N. Proposed Labeling:

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

O. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision