DxSelect™

(English)

REF EL1950-5
Rev. B

Enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of human antibodies to JC virus

For *in vitro* diagnostic use

INTENDED USE

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.

SUMMARY AND EXPLANATION OF THE TEST

Progressive multifocal leukoencephalopathy (PML) is an opportunistic infection of the central nervous system (CNS) caused by JC virus (JCV), a polyoma virus, that is pathogenic only in humans. It is thought that exposure to the JC virus occurs early in life (pre-adolescence) and recently published studies have reported that approximately 50% to 60% of adults have been infected with JCV, as evidenced by the presence of antibody to JCV in the serum.[1,2,3] In rare instances, the virus reactivates and progresses to PML such as in individuals who are immune compromised.[4,5,6] Treatment with immunomodulatory therapies increase the risk of developing PML in JCV infected individuals. While JCV exposure is necessary for the development of PML, the development of the disease is also dependent on both host and viral factors, as well as immune status.[9] Since JCV infection is a necessary step for PML development, an assay to detect JCV exposure in patients may be a potentially useful tool to stratify patients for PML risk (i.e., identifying patients who may be at a lower or higher risk of developing PML).

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 (Tysabri®) 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 **Table 1.**

Table 1: Estimated United States Incidence of PML Stratified by Risk Factor

Anti-JCV Antibody Negative**	TYSABRI Exposure†	Anti-JCV Antibody Positive*	
		No Prior Immunosuppressant Use	Prior Immunosuppressant Use
<1/1,000	1-24 months	<1/1,000	1/1,000
	25-48 months	3/1,000	13/1,000
	49-72 months	7/1,000	9/1,000

Notes: *Based on US postmarketing PML data as of September 3, 2013, and TYSABRI use data as of August 31, 2013.
Calculation based on 2 cases of anti-JCV antibody negative PML in patients exposed for at least 1 month of therapy as of September 3, **2013. Data for anti-JCV antibody negative patients reflects worldwide exposure.
†Data beyond 6 years of treatment are limited.
The anti-JCV antibody status was determined using an anti-JCV antibody test (ELISA) that has been analytically and clinically validated and is configured with detection and inhibition steps to confirm the presence of JCV-specific antibodies with an analytical false negative rate of 3%.

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.

MATERIALS SUPPLIED

The Focus Diagnostics STRATIFY JCV® DxSelect™ test kit contains the following components. Allow the supplied reagents to warm to 20 to 25°C before use. All un-opened materials are stable at 2 to 8°C until the expiration date stated on the reagent label. Opened materials are stable at 2 to 8°C for one month after opening or until expiration whichever comes first.

JCV Antigen Wells, 96 wells | **REF** | **EL1951** | **Ag**

Five 96-well plates. Each plate contains 12 eight-well polystyrene microwell strips on 1 frame. Each well is coated with recombinant JCV VLP (virus-like particles) antigen. To avoid condensation, allow the antigen strips to warm to 20 to 25°C before opening the sealed packets.

JCV Antigen, 0.05 mL | **REF** | **EL1952** | **Ag**

Five vials of recombinant JC VLP. Contains 0.1% sodium azide as a preservative. Requires dilution before use.

JCV Cut-Off Calibrator, 0.3 mL | **REF** | **EL1953** | **CONTROL** | **CAL**

Five vials of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use.

JCV Positive Control, 0.3 mL | **REF** | **EL1954** | **CONTROL** | **+**

Five vials of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use.

JCV Negative Control, 0.3 mL | **REF** | **EL1955** | **CONTROL** | **–**

Five vials of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use.

JCV Conjugate, 16 mL | **REF** | **EL1956** | **CONJ** | **Ab**

Five bottles of affinity-purified and peroxidase-conjugated donkey anti-human antibodies. Contains protein, buffer and preservatives. Ready to use.

Casein Sample Diluent, 112 mL | **REF** | **EL1957** | **DIL** | **SPE**

Five bottles of protein, surfactant, and non-azide preservatives in PBS. Ready to use.

TMB Substrate, 16 mL | **REF** | **EL1958** | **SUBS** | **TMB**

Five bottles of tetramethylbenzidine (TMB) and hydrogen peroxide in buffer. A dark blue color indicates contamination with peroxidase. If this occurs, use a fresh bottle. Ready to use.

10X Wash Buffer, 100 mL | **REF** | **EL0405** | **BUF** | **WASH**

Five bottles of surfactant in PBS with non-azide preservatives. Prepare 1X wash buffer solution before use.

To prepare a 1X wash buffer solution, mix 100 mL 10X Wash Buffer with 900 mL distilled or deionized water and rinse out any crystals that may be present. Use only the highest grade of distilled or deionized water for the preparation of the 1X wash buffer. Some sources of deionized water may contain materials, which can interfere with the assay. Swirl until well mixed and all crystals are dissolved.

Stop Reagent, 16 mL | **REF** | **EL0105** | **SOLN** | **STOP**

Five bottles of 1 M sulfuric acid. Ready to use.

Sealing Tape

15 sheets of sealing tape.

MATERIALS REQUIRED, BUT NOT SUPPLIED

1. Distilled or deionized water
2. 250 or 500 mL wash bottle
3. 1 L graduated cylinder
4. Borosilicate glass test tubes or equivalent
5. 10 µL and 100 µL pipettors with disposable tips (100 µL 8 or 12 -channel pipettor recommended for runs over 48 wells)

6. 1 mL pipet or dispenser
7. 10 and 25 mL pipet
8. Timer
9. Paper towels or absorbent paper
10. Sink or Discard Basin
11. Vortex mixer or equivalent
12. ELISA plate spectrophotometer, wavelength = 450 nm
13. Clean reagent trough or reservoir for each assay reagent.

SHELF LIFE AND HANDLING

1. Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2 to 8 °C.
2. Store reagents at 2 to 8°C.
3. Do not use test kit or reagents beyond their expiration dates.
4. Do not expose reagents to strong light during storage or incubation.
5. Allow reagents to warm to 20 – 25°C before use.
6. After initial use, return each component to refrigerator (2 to 8°C) and store for one month or until expiration date, whichever comes first.
7. Do not pour back used reagents into the original containers.
8. Do not use contaminated reagents.
9. Store reagents in their original containers.
10. Do not substitute or mix reagents from different kit lots or from other manufacturers.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antibody and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions. CDC and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.[7,8]
3. The antigen wells are produced with recombinant JC virus-like particles. After adding patient or control specimens, the strips should be considered potentially infectious and handled accordingly.
4. Sodium azide at a concentration of 0.1% has been added to some reagents as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, those reagents (see MATERIALS SUPPLIED, above) should be discarded into sewage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible. Occasionally decontaminate the drains with 10% sodium hydroxide (CAUTION: caustic), allow to stand for 10 minutes, then flush with large volumes of water.
5. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
6. Cross-contamination of patient specimens can cause erroneous results. Add patient specimens and handle strips carefully to avoid mixing of sera or plasma from adjoining incubation tray wells. Decant carefully.
7. Bacterial contamination of serum or plasma specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
8. Perform the assay at 20 to 25°C.
9. Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values.
10. The stop reagent contains sulfuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.
11. A dark blue color in the TMB Substrate indicates contamination with peroxidase. If this occurs, use a fresh bottle.

SPECIMEN COLLECTION AND PREPARATION

Serum and plasma are the specimen source. No attempt has been made to assess the assay's compatibility with other specimens. Do not use hyperlipemic, heat-inactivated, hemolyzed, or contaminated specimens, which may cause erroneous results. Samples containing antinuclear antibodies or rheumatoid factor have not been evaluated with this assay and may cause erroneous results.

Specimen Collection and Handling
Collect blood samples aseptically using approved venipuncture techniques by qualified personnel.[7]

For serum, allow blood samples to clot at room temperature prior to centrifugation. Plasma samples can be centrifuged immediately or within 2 hours of collection. Aseptically transfer serum or plasma to a tightly closing sterile container for storage.

Separated serum or plasma should remain at 22°C for no longer than 8 hours. If the assay will not be completed within 8 hours, refrigerate the sample at 2 to 8°C. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at –20°C or colder. Avoid repeated freeze/thaw of samples.[7] Thaw and mix samples well prior to use.

ASSAY PROCEDURE – DETECTION ASSAY

1. Bring all necessary reagents to 20 to 25°C before use. Remove the Antigen Wells packet from cold storage. To avoid condensation in the micro-well strips, allow the Antigen Wells packet to reach 20 to 25°C before opening the foil pouch. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and reseal completely. Store unused antigen wells at 2 to 8°C in the original foil

pouch. (Note: At the end of the assay, retain the frame for use with the remaining strips.)

2. Dilute each specimen, controls and calibrator 1:101. For example, label tubes and dispense 1 mL of Sample Diluent into each labeled tube. Add 10 µL of specimen, control or calibrator to each appropriate tube containing the 1 mL Sample Diluent and mix well by vortex mixing. All specimens and controls must be diluted once and run in duplicate, and the Cut-Off Calibrator must be diluted twice and run in duplicate for a total of 4 wells. See Quality Control section for more details.
3. Dispense 100 µL of the 1:101 dilution of specimens, controls and calibrator to the appropriate wells. See an example of a plate layout below.
4. Cover plates with sealing tape, and incubate for 60 ± 5 minute at 20 to 25°C.
5. Remove sealing tape, and empty the contents of the wells into a sink or a discard basin.
   Note: Do not reuse sealing tape.
6. Fill each well with a gentle stream of 1X Wash Buffer solution from a wash bottle then empty contents into a sink or a discard basin.
7. Repeat wash (step 6) an additional 2 times.
8. Tap the antigen wells vigorously to remove 1X Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual 1X Wash Buffer.
9. Dispense 100 µL Conjugate to all wells, using a 100 µL pipette.
10. Cover plates with sealing tape and incubate for 30 ± 2 minute at 20 to 25°C.
11. Repeat wash steps 5 through 8.
12. Dispense 100 µL of Substrate Reagent to all wells, using a 100 µL pipette. Begin incubation timing with the addition of TMB Substrate to the first well. (Note: Never pour the substrate reagent into the same trough as was used for the conjugate.)
13. Incubate for 20 ± 2 minute at 20 to 25°C.
14. Stop the reaction by adding 100 µL of Stop Reagent to all wells using a 100 µL pipette. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. Wells with a blue color will change to a yellow color.
15. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (Note: Large bubbles on the surface of the liquid may affect the OD readings.)
16. Measure the absorbance of each well within 15 minutes of stopping the assay. Set the ELISA plate spectrophotometer at a wavelength of 450 nm.

Example plate layout for Detection Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	C/O-1	C/O-1	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
B	C/O-2	C/O-2	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
C	PC	PC	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39
D	NC	NC	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32	Sample 40	Sample 40
E	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33	Sample 41	Sample 41
F	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34	Sample 42	Sample 42
G	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35	Sample 43	Sample 43
H	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36	Sample 44	Sample 44

PC = positive control; NC = negative control; C/O – cut-off calibrator

QUALITY CONTROL – DETECTION ASSAY

Each plate run (or strips from a single plate) must include the Cut-Off Calibrator and the two controls. If multiple plates are run, include the Cut-Off Calibrator and both controls on each plate. All specimens and controls must be diluted once and run in duplicate, and the Cut-Off Calibrator must be diluted twice and run in duplicate for a total of 4 wells.

The Cut-Off Calibrator has been formulated to give the optimum differentiation between negative and positive specimens. Although the absorbance value may vary between runs and between laboratories, the mean value for the Cut-Off Calibrator wells must be within 0.600 to 1.700 OD units. At least 3 of the 4 replicates of the Cut-Off Calibrator ODs must be within 0.100 absorbance units from the mean value. One replicate may be excluded; if a replicate is excluded the mean OD must be recalculated using the 3 acceptable values.

Results are reported as index values relative to the Cut-Off Calibrator. To calculate index values, divide the controls or specimen mean OD value by the Cut-Off Calibrator mean OD value.

Note: Some brands of ELISA plate spectrophotometers may output non-numeric results instead of OD values for measurements above the limit of detection of the spectrophotometer. Under such circumstances, the index values cannot be determined. A specimen with OD values or result indicating greater than the spectrophotometer range is positive for the presence of antibodies to JCV.

Run Validity

1. Calculate the %CV for OD values for Positive Control and Negative Control.
   a. The %CV for OD values for Positive Control and Negative Control must be less than or equal to 20%.
2. The Positive Control index value must be between 0.90 and 1.70.
3. The Negative Control index value must be less than 0.20.

If the Cut-Off Calibrator or controls are not within these parameters, specimen test results should be considered invalid and the assay repeated. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Specimen Validity

1. Calculate the %CV for OD values for each specimen.
    a. The %CV for OD values for each specimen must be less than or equal to 20%.
2. If the %CV for OD values for each specimen is greater than 20%, the specimen should be retested.

INTERPRETATION OF TEST RESULTS – DETECTION ASSAY

Calculate the index value for all specimens and report results as indicated in the table below.

To calculate the index value, divide the mean OD of the specimen by the mean OD of the Cut-Off Calibrator.

Index	Interpretation	Recommendation
>0.40	**Positive**	A specimen with an index value of > 0.40 is positive for the presence of antibodies to JCV.
≥0.20 and ≤0.40	**Indeterminate:**	A specimen with an index value of ≥0.20 but ≤0.40 is considered an indeterminate result. Specimens with indeterminate results in the ELISA are evaluated in the confirmation assay (inhibition assay) and final results should be reported from the confirmation assay analysis.
<0.20	**Negative***	An index value of < 0.20 indicates that no antibodies to JCV were detected.

*** Please note that periodic retesting of patients who test JCV antibody negative is recommended.[9]**

ASSAY PROCEDURE - CONFIRMATION (INHIBITION) ASSAY

1. Run the confirmation assay for all samples that produce an "Indeterminate" result from the Detection Assay. Each specimen will be diluted in Sample Diluent (uninhibited specimen) and in Confirmation Diluent (inhibited specimen.)
2. Bring all necessary reagents to 20 to 25°C before use. Remove the Antigen Wells packet from cold storage. To avoid condensation, allow micro-well strips to reach 20 to 25°C before opening the foil pouch. If less than a full plate is to be used, return unused strips to the foil pouch with desiccant and reseal completely. Store unused antigen wells at 2 to 8°Cin the original foil pouch. (Note: At the end of the assay, retain the frame for use with the remaining strips.)
3. Prepare Confirmation Diluent.
    a. Prepare a 1:2500 dilution of JCV Antigen into Sample Diluent. For example, add 10 uL of the JCV Antigen into 25 mL of Sample Diluent or 5 uL of the JCV Antigen into 12.5 mL of Sample Diluent.
        Note: JCV Antigen should <u>not</u> be vortexed. The JCV Antigen solution should be inverted several times to thoroughly mix.
4. Prepare, as in the Detection Assay, the uninhibited specimens, controls and calibrator by diluting 1:101 with Sample Diluent. For example, label tubes and dispense 1 mL of Sample Diluent into each labeled tube. Add 10 µL of specimen, control or calibrator to each appropriate tube containing the 1 mL Sample Diluent and mix well by vortex mixing. All uninhibited specimens and uninhibited controls must be diluted once and run in duplicate, and the uninhibited Cut-Off Calibrator must be diluted twice and run in duplicate for a total of 4 wells. See Quality Control section for more details.
5. Prepare the inhibited Positive Control (PC-I) and inhibited specimens by diluting 1:101 with Confirmation Diluent. For example, label tubes and dispense 1 mL of Confirmation Diluent into each labeled tube. Add 10 µL of Positive Control or specimen to each appropriate tube containing the 1 mL Confirmation Diluent and mix well by pipetting. **<u>Do not vortex</u>** solutions containing JCV Antigen. The inhibited Positive Control and inhibited specimens must be diluted once and run in duplicate. See Quality Control section for more details.
6. After the last sample is diluted, allow all samples to sit for a minimum of 10 minutes to a maximum of 20 minutes at 20 to 25°C before running the assay.
7. Dispense 100 µL of the 1:101 dilution of uninhibited calibrator, controls and specimens to the appropriate wells. See an example of a plate layout below.
8. Dispense 100uL of the 1:101 dilution of inhibited Positive Control (PC-I) and inhibited specimens to the appropriate wells. See an example of a plate layout below.
9. Cover plates with sealing tape, and incubate for 60 ± 5 minute at 20 to 25°C.
10. Remove sealing tape, and empty the contents of the wells into a sink or a discard basin.
    Note: Do not reuse sealing tape.
11. Fill each well with a gentle stream of 1X Wash Buffer solution from a wash bottle then empty contents into a sink or a discard basin.
12. Repeat wash (step 11) an additional 2 times.
13. Tap the antigen wells vigorously to remove the 1X Wash Buffer. Blot the emptied Antigen Wells face down on clean paper towels or absorbent paper to remove residual 1X Wash Buffer.
14. Dispense 100 µL of Conjugate to all wells, using a 100 µL pipette.
15. Cover plates with sealing tape and incubate for 30 ± 2 minute at 20 to 25°C.
16. Repeat wash steps 10 through 13.
17. Dispense 100 µL of Substrate Reagent to all wells, using a 100 µL pipette. Begin incubation timing with the addition of TMB Substrate to the first well. (Note: Never pour the substrate reagent into the same trough as was used for the conjugate.)
18. Incubate for 20 ± 2 minute at 20 to 25°C.
19. Stop the reaction by adding 100 µL of Stop Reagent to all wells using a 100 µL pipette. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. Wells with a blue color will change to a yellow color.
20. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (Note: Large bubbles on the surface of the liquid may affect the OD readings.)
21. Measure the absorbance of each well within 15 minutes of stopping the assay. Set the ELISA plate spectrophotometer at a wavelength of 450 nm.

Example plate layout for Confirmation Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	C/O-1	C/O-1	Sample 5	Sample 5	Sample 13	Sample 13	(empty)	(empty)	Sample 5-I	Sample 5-I	Sample 13-I	Sample 13-I
B	C/O-2	C/O-2	Sample 6	Sample 6	Sample 14	Sample 14	(empty)	(empty)	Sample 6-I	Sample 6-I	Sample 14-I	Sample 14-I
C	PC	PC	Sample 7	Sample 7	Sample 15	Sample 15	PC-I	PC-I	Sample 7-I	Sample 7-I	Sample 15-I	Sample 15-I
D	NC	NC	Sample 8	Sample 8	Sample 16	Sample 16	(empty)	(empty)	Sample 8-I	Sample 8-I	Sample 16-I	Sample 16-I
E	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 1-I	Sample 1-I	Sample 9-I	Sample 9-I	Sample 17-I	Sample 17-I
F	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 2-I	Sample 2-I	Sample 10-I	Sample 10-I	Sample 18-I	Sample 18-I
G	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 3-I	Sample 3-I	Sample 11-I	Sample 11-I	Sample 19-I	Sample 19-I
H	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 4-I	Sample 4-I	Sample 12-I	Sample 12-I	Sample 20-I	Sample 20-I
	Cut-offs, Controls and Samples diluted in Sample Diluent (Uninhibited controls and samples)						Positive Control and Samples diluted in Confirmation Diluent (Inhibited controls and samples)					

QUALITY CONTROL - CONFIRMATION (INHIBITION) ASSAY

Each plate run (or strips from a single plate) must include the uninhibited Cut-Off Calibrator and the two uninhibited controls. If multiple plates are run, include the uninhibited Cut-Off Calibrator and both uninhibited controls on each plate. All uninhibited specimens and uninhibited controls must be diluted once and run in duplicate, and the uninhibited Cut-Off Calibrator must be diluted twice and run in duplicate for a total of 4 wells. The inhibited Positive Control (PC-I) must also be diluted once and run in duplicate on each plate. All inhibited specimens must be diluted once and run in duplicate.

The Cut-Off Calibrator has been formulated to give the optimum differentiation between negative and positive specimens. Although the absorbance value may vary between runs and between laboratories, the mean value for the Cut-Off Calibrator wells must be within 0.600 to 1.700 OD units. At least 3 of the 4 replicates of the Cut-Off Calibrator ODs must be within 0.100 absorbance units from the mean value. One replicate may be excluded; if a replicate is excluded the mean OD must be recalculated using the 3 acceptable values.

Results are reported as % inhibition. Please see Interpretation of Test Results – Confirmation Assay (Inhibition Assay) section on how to calculate % inhibition.

Note: Some brands of ELISA plate spectrophotometers may output non-numeric results instead of OD values for measurements above the limit of detection of the spectrophotometer. Under such circumstances, the % inhibition cannot be determined. A specimen with a result of an inhibited OD value greater than the spectrophotometer range indicates no antibodies to JCV were detected. No further confirmatory testing is required.

Run Validity

1. Calculate the %CV for OD values for uninhibited Positive Control, inhibited Positive Control (PC-I) and uninhibited Negative Control.
    a. The %CV for OD values for uninhibited Positive Control, inhibited Positive Control (PC-I) and uninhibited Negative Control must be less than or equal to 20%.
2. The Positive Control uninhibited index value must be between 0.90 and 1.70.
3. The Positive Control % inhibition must be greater than 70%.
4. The Negative Control index value must be less than 0.20.

If the Cut-Off Calibrator or controls are not within these parameters, specimen test results should be considered invalid and the assay repeated. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Specimen Validity

1. Calculate the %CV for uninhibited and inhibited OD values for each specimen.
    a. The %CV for uninhibited and inhibited OD values for each specimen must be less than or equal to 20%.
2. If the %CV for uninhibited or inhibited OD values for each specimen is greater than 20%, the specimen should be retested.

INTERPRETATION OF TEST RESULTS – CONFIRMATION (INHIBITION) ASSAY

Calculate the percent inhibition (% inhibition) for all specimens and report the results as indicated in the table below.

To calculate the % inhibition, first calculate the uninhibited mean OD and the inhibited mean OD for the specimen. The % inhibition is calculated as follows:

[(uninhibited mean OD – inhibited mean OD)/uninhibited mean OD]*100 = % inhibition

Report the % inhibition rounded to one decimal place (For example, 40.357% will be reported as 40.4%).

% Inhibition	Interpretation	Recommendation
>45.0%	Positive	A specimen with a % inhibition value of > 45.0% is positive for the presence of antibodies to JCV. No further confirmatory testing is required.
≤45.0%	Negative*	A specimen with a % inhibition value of ≤ 45.0% indicates that no antibodies to JCV were detected. If % inhibition for a specimen is negative, then the sample is reported as Negative. No further confirmatory testing is required.

*** Please note that periodic retesting of patients who test JCV antibody negative is recommended.[9]**

ESTABLISHMENT OF THE CUT POINT

To characterize antibody responses against infectious agents in humans, it is critical to have reference sera from both infected and non-infected individuals. However, it should be noted that JCV infection is unusual in that it 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[9]. 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.

LIMITATIONS

1. The performance characteristics of the assay were based on testing samples from Multiple Sclerosis patients who were naïve to treatment or being treated with natalizumab.
2. The performance of this assay has not been established for matrices other than serum or plasma (EDTA, sodium heparin).
3. All results from this and other serology assays must be correlated with clinical history, epidemiological data, and other data available to the attending physician in evaluating the patient.
4. The physician should consider all known risk factors when using JCV serological status to aid in evaluating the risk of developing PML.
5. Patients who are anti-JCV antibody negative are still at risk for the development of PML due to the potential for a new JCV infection or a false negative test result. The reported rate of seroconversion in patients with MS (changing from anti-JCV antibody negative to positive and remaining positive in subsequent testing) is 3 to 8 percent annually. In addition, some patients' serostatus may change intermittently
6. Negative results do not rule out the presence of the JC virus (level of antibodies may have been transiently decreased in blood under circumstances such as immune suppression or may be below the level of detection in the assay)
7. The limit of detection (cut-off) was set using sera from individuals with positive JCV urinary DNA testing, and is assumed to be applicable to individuals who do not shed JCV DNA in urine,
8. Testing should not be performed for at least two weeks following plasma exchange due to removal of antibodies from the serum.
9. Gamma globulin is known to cross react with this assay. Patients undergoing γ globulin therapy may have erroneous results.
10. Samples containing antibodies to *C. pneumonia*, CMV, HIV or HSV-1 may cross react with this assay.
11. Potential cross reactivity with antibodies to other polyoma viruses (Merkel Cell virus, WU virus or KI virus) has not been evaluated.
12. The performance of this assay has not been evaluated with samples that contain rheumatoid, antinuclear antibody or has bacterial contamination. These substances may interfere with assay performance.
13. Assay reagents from one kit lot cannot be used with reagents from another kit lot.
14. This assay should not be used for the diagnosis of PML nor to assess the treatment efficacy with natalizumab.
15. High positives may result in an OD that is higher than the reading range of the spectrophotometer in which a numerical OD value will not be given and an index value will not be calculated by the reader. Each laboratory should determine the reporting range for their index values. When the OD is higher than the range, then the index value can be reported as greater than the top of the range.
16. The prevalence of JCV specific antibodies in the test population will affect the assay's predictive value.
17. A single positive result only indicates previous immunologic exposure; level of antibody response may not be used to determine active infection or disease stage.
18. The magnitude of the index value above the Cut-Off does not indicate the total amount of antibody present.

PERFORMANCE CHARACTERISTICS

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 1: 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 natalizumab ≥ 18 months for Positive result		5.09 95% CI: 3.70 to 7.01		
Risk of PML (per 1,000) treated with natalizumab ≥ 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 a cleared Anti-JCV 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. Positive Percent Agreement was greater than 97% for each group and Negative Percent agreement was greater than 90% for each group.

Table 2: Agreement for Clinical Samples – Patients Receiving Natalizumab

STRATIFY JCV® DxSelect™	Cleared Anti-JCVAssay		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 3: Agreement for Clinical Samples - Patients Considering Natalizumab

STRATIFY JCV® DxSelect™	Cleared Anti-JCV Assay		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%		

Positivity Rate and Expected Values:

The STRATIFY JCV® DxSelect™ assay was 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) and 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 Table 5. 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% which is consistent with what has been reported in the literature. [9, 10] JCV antibody positivity rate 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.[10,3, 2]

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 4: 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[1] (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)

1. A total of 10.4% of the samples tested in the AFFIRM study and 16.4% of the samples tested in the STRATIFY-2 study were indeterminate in the DETECTION ASSAY.

Reproducibility

The reproducibility of the assay was assessed using a protocol based on a CLSI guideline for estimating precision of an assay[11]. The protocol consisted of testing three replicates of each panel member for two runs a day for a total of five days at two external testing sites and one internal site. The reproducibility testing panel consisted of four levels of sera and four levels of plasma (EDTA) samples and the low and high positive controls. The four levels included a negative, low, indeterminate and moderate positive sample prepared in a serum matrix and a negative, low, indeterminate and moderate positive sample prepared in a plasma matrix. The indeterminate samples were tested simultaneously in the Confirmation Assay and the Detection Assay.

Table 5: Reproducibility

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
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
%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 at the Lower Cut Point

In order to demonstrate precision near the lower cut point, two contrived samples, one sera and one plasma (EDTA) containing the analyte at concentration near the lower cut-point of the assay. Each sample was diluted twenty times and tested at one internal site in the Detection Assay and the Confirmation Assay. As depicted in the following table the %CV was ≤ 2.6%.

Table 6: Reproducibility at the Lower Cut Point

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

Cross Reactivity

Cross reactivity was evaluated in a three part study conducted at an internal testing site. Part one of the 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 limit of detection 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 part one of the study.

Table 7: Cross Reactivity - Part One – Spiked Antibodies

Cross Reactant	Concentration	Number 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 part two of the study the study panel consisted of a least twenty remnant specimens that previously tested positive for each potential cross reacting antibody. Each member of the panel was evaluated using the STRATIFY JCV® DxSelect™ assay 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 (>70%) it may be an indicator of potential cross reactivity. Four groups of patients (*C. pneumoniae*, HIV, CMV, 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 8: 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
	HHV 6	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

Part three 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 BKV 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 JCV VLP and BKV VLP. A control set consisted of samples spiked with JCV VLP at the same concentration of VLP that is used in the confirmation assay. The test samples were spiked with BKV 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 BKV 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 BKV VLP that ranged from -15 to 27%. No samples exhibited

>45% change in the OD signal when spiked with BKV VLP, indicating that the assay does not cross react with BKV.

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 were evaluated using a protocol based on a CLSI guideline for estimating the effect of interfering substances.[12] The testing panel consisted of sera and plasma samples that contain JCV antibody with an index value that is close to the assay cut off. The samples are spiked with the potential interferent at the highest possible endogenous level and compared to baseline testing of the same serum and plasma samples that did not contain the interferent. 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, since the seroprevalence of antibodies to JCV virus is approximately 55% in the normal population it is expected to react with this assay.

Table 9: 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 dilution of Gamma Globulin stock solution exhibited Index values - 3.22, 3.57 and 3.40.

HOOK EFFECT

The Hook Effect was investigated during the optimization of the JCV VLP coating concentration and conjugate dilution using a panel of serum samples ranging from negative reactivity to strong positive reactivity (beyond the limit of the plate reader at OD_{450} – 4.000) to JCV. No Hook Effect was observed at the optimal coating VLP concentration and conjugate dilution for this assay using the panel tested.

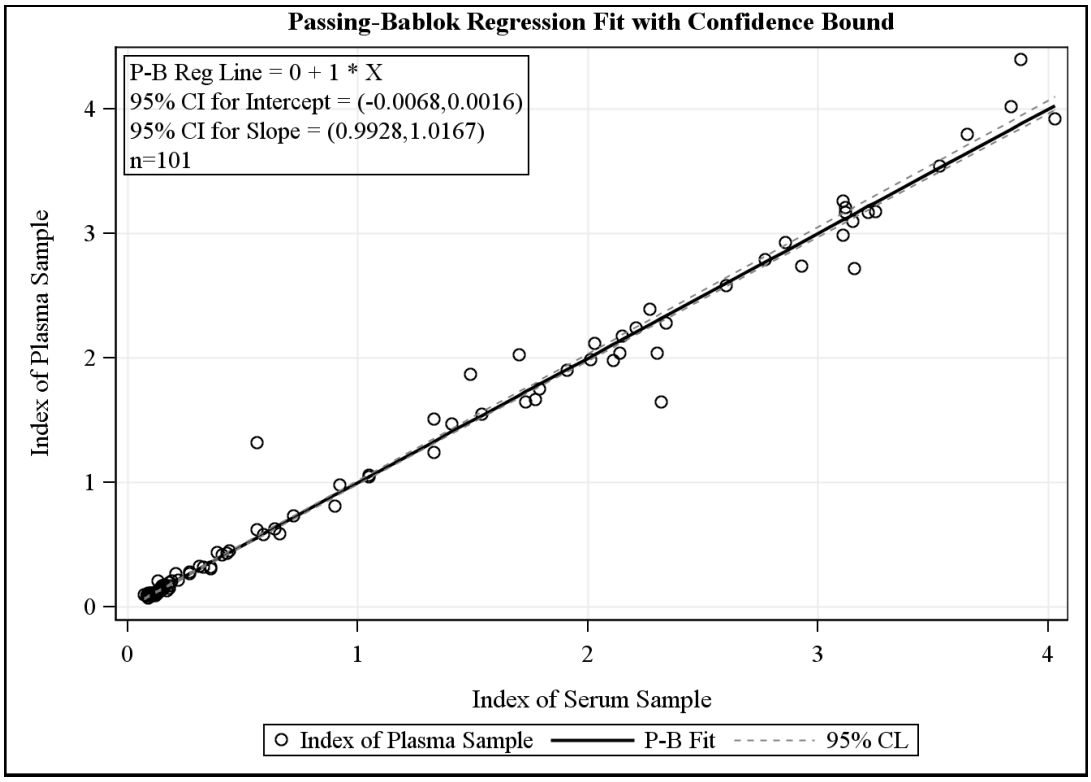
SAMPLE MATRIX COMPARISON

A panel of 109 paired sera and plasma samples (EDTA) 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 10: 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 1: Passing-Bablok Regression Analysis (Sera vs. Plasma)



Sample Comparison -Fresh vs. Frozen

A panel of 53 pairs of fresh and frozen plasma specimens (EDTA) and 53 pairs of fresh and frozen sera specimens were evaluated in the STRATIFY JVC DxSelect assay. Of the total 106 pairs, two serum pairs and three plasma pairs were excluded from the regression analysis due to having OD readings above the spectrophotometers measuring range. One pair of serum samples was excluded from the regression analysis due to an invalid result for the fresh specimen. Passing-Bablok regression analysis of the pairs of fresh vs. frozen specimens demonstrates a slope of 1 with a 95% CI of (0.9900 to 1.0221) and an intercept of 0 with a 95% CI of (-0.0090 to 0.0028). Analysis of the qualitative results yielded a Positive Percent Agreement = 96% (72/75), 95% CI: (88.9 to 98.6%) and Negative Percent Agreement = 96.7% (29/30), 95%CI: (83.3 to 99.4%).

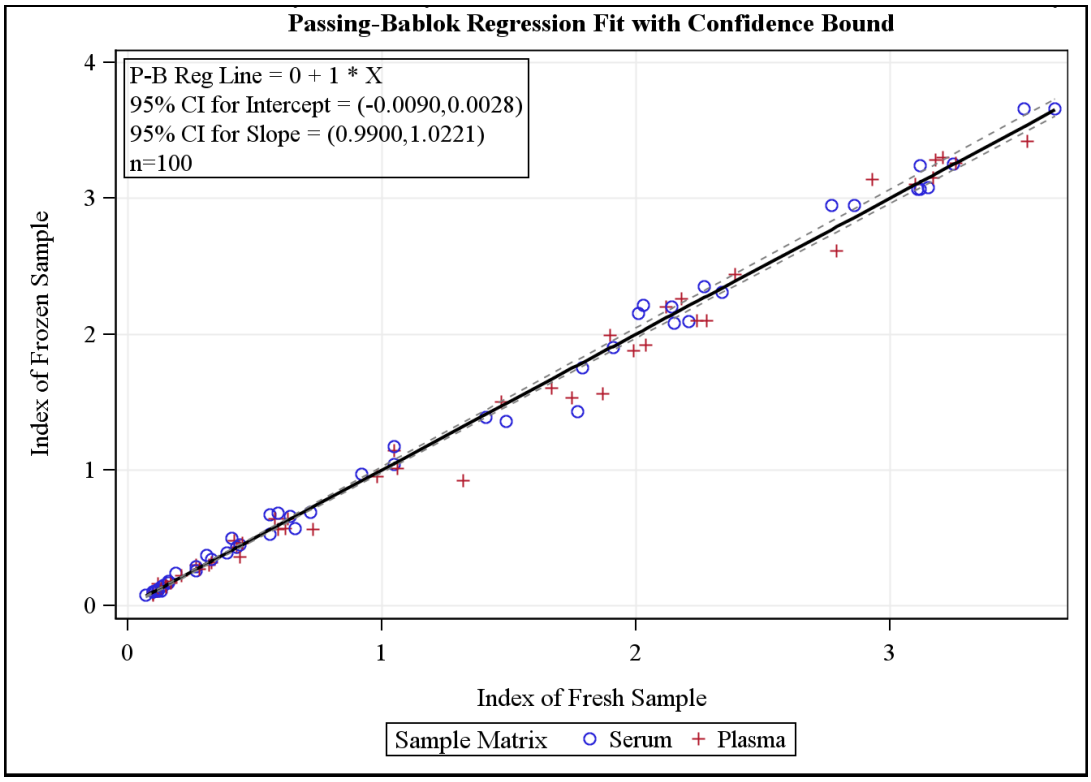
Table 11: Sample Comparison -Fresh vs. Frozen

		Final Frozen Result (Count)		
Sample Matrix	**Final Fresh Result**	**ND**	**D**	**All**
Plasma	**ND**	16		16
	D	1	36	37
	All	17	36	53
Serum	**ND**	13	1	14
	D	2	36	38
	NV	1		1
	All	16		16
All		32	68	106

ND = Not Detected, D = Detected; NV = Invalid

* Three plasma pairs and two serum pairs had results that were above the range of the spectrophotometer, these samples are considered detected.

Figure 2: Sample Comparison –Regression Analysis Fresh vs. Frozen



Note: Two serum pairs and three plasma pairs were excluded from the regression analysis due to having OD readings above the spectrophotometers measuring range. One pair of serum samples was excluded from the regression analysis due to an invalid result for the fresh specimen.

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