EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Ventana Kappa and Lambda Dual ISH mRNA Probe Cocktail
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN240025

B Applicant

Ventana Medical Systems, Inc.

C Proprietary and Established Names

Ventana Kappa and Lambda Dual ISH mRNA Probe Cocktail

D Regulatory Information

Product Code	Classification	Regulation Section	Panel
SDP	Hematolymphoid neoplasm immunoglobulin mRNA in situ hybridization detection device	21 CFR 864.1861	Hematology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for Kappa and Lambda Dual ISH mRNA Probe Cocktail Assay

B Measurand:

Kappa and Lambda mRNA ratios

C Type of Test:

In situ hybridization (ISH) for detection of Kappa/Lambda ratios in B-cell and plasma cell neoplasms.

III Indications for Use:

A Indication(s) for Use:

The VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail Assay is a qualitative assay that is used to detect the expression of kappa and lambda immunoglobulin light chains in formalin-fixed paraffin embedded (FFPE) human hematolymphoid specimens by in situ hybridization (ISH).

The assay is intended as an aid in the diagnosis of mature B-cell lymphomas and plasma cell neoplasms. The VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail is indicated for use when a biopsy of lymph node or bone marrow (core biopsy and clot section) indicates inconclusive results. It enables the assessment of both markers in the context of one another on a single slide as an aid in differentiating between a reactive process or B-cell lymphoma and plasma cell neoplasms.

This is not a standalone test, and results should be evaluated by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

This product is intended for in vitro diagnostic (IVD) use.

B Special Instrument Requirements:

BenchMark ULTRA instrument

IV Device/System Characteristics:

A Device Description:

The VENTANA Kappa and Lambda Dual ISH mRNA Probe Cocktail (hereafter referred to as VENTANA K/L Probe Cocktail) is a qualitative mRNA reagent designed to detect expression of kappa and lambda immunoglobulin light chains in FFPE human lymphoid and bone marrow tissue by in situ hybridization (ISH). The VENTANA K/L Probe Cocktail contains sufficient reagent for 30 tests. One 6 mL dispenser contains approximately 0.120 µg/mL labeled probe in a formamide-based hybridization buffer (approximately 50% CH3NO). The kit contents are listed in Table 1. Materials Required but Not Provided are shown in Table 2.

Table 1. VENTANA K/L Probe Cocktail

Component	Configuration	Storage	Content
VENTANA K/L Probe Cocktail	1 dispenser x 6 ml	Store at 2 – 8°C. Do not freeze.	mRNA reagent in formamide buffer for detection of kappa and lambda mRNA

Table 2. Materials Required but Not Provided

VENTANA U6 BF Probe (Cat. No. 760-7062 / 08773866001)	Store at 2 – 8°C. Do not freeze.
ISH Negative Control (Cat. No. 780-2902 / 05272165001)	
VENTANA Silver ISH BF Detection Kit (Cat. No. 760-513 / 08507031001)	
VENTANA Magenta ISH DIG Detection Kit (Cat. No. 760-514 / 08507201001)	

B Principle of Operation

The VENTANA K/L Probe Cocktail is a qualitative mRNA reagent used in laboratories to detect expression of Kappa and lambda immunoglobulin light chains in FFPE human bone marrow and lymphoid tissue by in situ hybridization (ISH). The kit is a dual color test, using kappa and lambda oligonucleotide probes cocktailed into a single dispenser automated on the BenchMark ULTRA instrument. It enables the clinician to assess both markers in the context of one another on a single slide as an aid in differentiating between a reactive process or B-cell lymphoma and plasma cell neoplasms. Kappa target will stain magenta, and lambda target will stain black. The typical positive staining pattern for B-cells is a partial to full ring of punctate cytoplasmic staining, while plasma cells typically exhibit complete filling of the cytoplasm due to abundant mRNA.

Interpretation of Staining

The staining pattern is interpreted as a ratio of kappa to lambda expressing cells for the determination of restriction status. The normal immune response typically produces a kappa-heavy polyclonal population of approximately 2-3:1 kappa to lambda. For VENTANA K/L Probe Cocktail, a ratio greater than 4:1 is interpreted as kappa restricted, and a ratio lower than 1:2 is interpreted as lambda restricted (see Table 3).

Table 3. Scoring Criteria for determination of restriction status

Clinical Status	***KAPPA:LAMBDA* Ratio**
KAPPA Restricted	≥ 4:1
Non-restricted (in tissue sections on glass slides)	< 4:1, >1:2
LAMBDA Restricted	≤ 1:2

C Instrument Description Information

1. Instrument Name:
   BenchMark ULTRA instrument
   Host software: Ventana System Software (VSS) version 12.5.4

2. Specimen Identification:

   Specimen Preparation
   The VENTANA K/L Probe Cocktail is for use on human bone marrow and lymphoid FFPE tissue. Bone marrow specimens include clot sections and core biopsies for plasma cell neoplasm and clot sections only for B-cell neoplasm. Lymphoid tissue includes excisional and small biopsies. It is anticipated that the type and time of sample fixation may affect the performance of this assay and this impact was characterized. In addition, the type of decalcification procedure used on bone marrow core biopsies can also affect assay performance and this impact was characterized. Routinely processed FFPE tissues are suitable for use with VENTANA K/L Probe Cocktail. The recommended tissue fixative is 10% neutral buffered formalin (NBF) for 6-72 hours. Specimens should be cut to 4µm sections and placed on positively charged microscope slides. Slides should be stained immediately, as quality of RNA targets in cut tissue sections may diminish over time.

   For tissues that require a decalcification step, VENTANA K/L Probe Cocktail has been shown to be compatible with HCl, formic acid, and EDTA decalcification reagents, but this compatibility is highly dependent on reagent concentration and treatment time.

3. Quality Control:

Positive Control Tissue
It is recommended that a laboratory-specific tonsil control tissue should be included on each patient slide to ensure that the assay is performing as expected. Normal tonsil exhibits the full range of kappa and lambda expression, whereas the stroma serves as a negative staining element. Markedly diminished signal or excessive background indicates that an error may have occurred on that slide, and the slide should not be evaluated.

RNA Integrity Marker
The VENTANA K/L Probe Cocktail may have reduced performance in tissues where mRNA integrity has been impacted. Because RNA is susceptible to degradation, the ubiquitously expressed U6 transcript is commonly used as a surrogate to assess target degradation in tissue samples. Negative staining with VENTANA U6 BF Probe indicates that a new patient sample may be necessary. Patient samples stained with VENTANA U6 BF Probe should be run with the same staining procedure and pre-treatment selectable as were used for VENTANA K/L Probe Cocktail testing.

Negative reagent control
ISH Negative Control may be used in place of VENTANA K/L Probe Cocktail to assess for detection-driven background in a patient sample.

V Performance Characteristics:

A Analytical Performance:

1. Precision/Reproducibility:

Interlaboratory Reproducibility
This study was conducted to evaluate the interlaboratory reproducibility of the VENTANA K/L Probe Cocktail, as used on the BenchMark ULTRA instrument in the determination of Kappa/Lambda (K/L) restriction status [Kappa (K)-, Lambda (L)-, or non-restricted] in formalin-fixed, paraffin-embedded (FFPE) human bone marrow and lymphoid tissue. A total of 28 study cases selected were stained with the VENTANA K/L Probe Cocktail at each of 3 external clinical laboratories on each of 5 non-consecutive staining days using the same lots. Slides from each of the 28 study cases were stained with the VENTANA K/L Probe Cocktail at each of 3 study sites on each of 5 staining days, producing 420 K/L case slides read by 2 pathologists with a total of 840 possible observations.

The performance of the VENTANA K/L Probe Cocktail was to be considered acceptable if the 95% lower bound confidence interval (LBCI) for the percent agreement rate for each K/L restriction status across all observations was at least 80%.

As shown in Table 4, the Kappa Restricted Percent Agreement (KPRA), Lambda Restricted Percent Agreement (LRPA), and Non-Restricted Percent Agreement (NRPA) LBCIs for the primary analysis were 97.5%, 98.8%, and 96.1%, respectively, exceeding the minimum acceptable rate of 80% specified for each in the study protocol. The Overall Percent

Agreement (OPA) combines the KRPA, LRPA, and NRPA for the primary analysis, site-stratified analysis (all sites), and reader-stratified analysis (all readers) was 98.5% with a 95% CI of 97.7% to 99.2%.

Table 4. Overall concordance results by restriction status

Overall, Site-Stratified, and Reader-Stratified Agreement of K/L Status with the Case-Level Mode						
Rate	**Co-Primary Analysis**		**Site-Stratified Analysis (All Sites)**		**Reader-Stratified Analysis (All Readers)**	
	% (n/N)	**95% CI***	**% (n/N)**	**95% CI***	**% (n/N)**	**95% CI***
KRPA	98.8 (237/240)	97.5, 99.6	98.8 (237/240)	97.5, 99.6	98.8 (237/240)	97.5, 99.6
LRPA	99.6 (239/240)	98.8,100.0	99.6 (239/240)	98.8, 100.0	99.6 (239/240)	98.8, 100.0
NRPA	97.5 (351/360)	96.1, 99.0	97.5 (351/360)	96.1, 99.0	97.5 (351/360)	96.1, 99.0
OPA	98.5 (827/840)	97.7, 99.2	98.5 (827/840)	97.7, 99.2	98.5 (827/840)	97.7, 99.2

* Two-sided 95% CI calculated using the percentile bootstrap method with 2000 replicates or, for estimates of 100%, a Wilson score-based method.

Additionally, the average pairwise agreement rate for each restriction status between-site, between-readers, and between-days were reported (Table 5). Precision results for each sample were reported (Table 6) and demonstrate that the mean ratios fall within the expected restriction category.

Table 5. Results for overall between-site, between-reader, and between-day pairwise agreements for restriction status.

Pairwise Factor	Restriction Category	Agreement	95% CI
Between Sites	Kappa Restricted	95.6%	89.3, 98.1
	Lambda Restricted	98.8%	96.2, 100.0
	Non-restricted	96.6%	94.2, 98.4
	OPA	96.9%	95.2, 98.6
Between Readers	Kappa Restricted	95.5%	89.2, 98.1
	Lambda Restricted	98.8%	96.2, 100.0
	Non-restricted	96.6%	94.2, 98.4
	OPA	96.9%	95.1, 98.6
Between Days	Kappa Restricted	95.5%	89.2, 98.1
	Lambda Restricted	98.8%	96.2, 100.0
	Non-restricted	96.6%	94.2, 98.4
	OPA	96.9%	95.1, 98.6

Table 6. Precision results by sample

	Kappa/Lambda Status					Kappa/Lambda Derived Ratio					
Case #	**N**	**Case-Level Mode Status**	**Kappa Restricted**	**Lambda Restricted**	**Non-Restricted**	**N**	**Mean**	**Median**	**Min, Max**	**SD**	**%CV**
			N (%)								
1	30	Kappa Restricted	29 (96.7)	0	1 (3.3)	30	83.92	99.00	3.55, 100.00	32.10	38.26
2	30	Kappa Restricted	29 (96.7)	0	1 (3.3)	30	40.39	9.00	2.70, 100.00	42.93	106.29
3	30	Kappa Restricted	30 (100.0)	0	0	30	91.33	99.00	4.88, 100.00	24.70	27.05

Case #	Kappa/Lambda Status					Kappa/Lambda Derived Ratio					
	N	Case-Level Mode Status	Kappa Restricted	Lambda Restricted	Non-Restricted	N	Mean	Median	Min, Max	SD	%CV
			N (%)								
4	30	Kappa Restricted	30 (100.0)	0	0	30	82.87	99.00	9.00, 100.00	32.97	39.78
5	30	Kappa Restricted	30 (100.0)	0	0	30	36.70	19.00	9.00, 99.00	38.56	105.07
6	30	Kappa Restricted	30 (100.0)	0	0	28	89.42	99.00	9.00, 100.00	25.04	28.00
7	30	Kappa Restricted	30 (100.0)	0	0	25	97.20	99.00	49.00, 100.00	10.05	10.34
8	30	Kappa Restricted	29 (96.7)	1 (3.3)	0	27	89.73	99.00	0.43, 100.00	27.70	30.87
9	30	Lambda Restricted	0	30 (100.0)	0	30	0.01	0.01	0.01, 0.10	0.02	122.47
10	30	Lambda Restricted	0	30 (100.0)	0	30	0.12	0.11	0.01, 0.43	0.13	112.14
11	30	Lambda Restricted	0	30 (100.0)	0	30	0.02	0.01	0.01, 0.11	0.02	135.30
12	30	Lambda Restricted	0	30 (100.0)	0	30	0.02	0.01	0.01, 0.11	0.03	141.02
13	30	Lambda Restricted	0	29 (96.7)	1 (3.3)	30	0.21	0.01	0.01, 3.76	0.68	331.71
14	30	Lambda Restricted	0	30 (100.0)	0	30	0.01	0.01	0.00, 0.01	0.00	18.89
15	30	Lambda Restricted	0	30 (100.0)	0	30	0.04	0.01	0.01, 0.18	0.05	118.71
16	30	Lambda Restricted	0	30 (100.0)	0	30	0.01	0.01	0.00, 0.05	0.01	82.40
17	30	Non-restricted	2 (6.7)	0	28 (93.3)	30	2.54	3.00	1.50, 4.88	0.91	35.99
18	30	Non-restricted	1 (3.3)	0	29 (96.7)	30	2.26	2.10	1.00, 4.88	0.99	43.90
19	30	Non-restricted	1 (3.3)	0	29 (96.7)	30	2.06	1.50	1.00, 9.00	1.55	75.09
20	30	Non-restricted	0	0	30 (100.0)	30	1.85	1.50	1.00, 3.76	0.90	48.69
21	30	Non-restricted	0	0	30 (100.0)	30	1.73	1.50	1.00, 3.00	0.62	35.81
22	30	Non-restricted	2 (6.7)	0	28 (93.3)	30	2.19	1.50	1.00, 5.67	1.25	57.06
23	30	Non-restricted	0	0	30 (100.0)	30	2.13	1.50	1.00, 3.76	0.90	42.44
24	30	Non-restricted	1 (3.3)	0	29 (96.7)	30	2.34	2.23	1.00, 4.88	1.02	43.80
25	30	Non-restricted	0	0	30 (100.0)	30	1.40	1.00	0.67, 2.70	0.59	42.38
26	30	Non-restricted	0	0	30 (100.0)	30	1.79	1.50	1.00, 3.35	0.79	44.38
27	30	Non-restricted	1 (3.3)	1 (3.3)	28 (93.3)	30	1.86	1.50	0.18, 4.88	1.06	56.90
28	30	Non-restricted	0	0	30 (100.0)	30	1.38	1.00	0.82, 3.00	0.63	46.10

Cocktail across three lots on formalin-fixed paraffin embedded tissues. Eight kappa restricted, 8 lambda restricted, and 10 non-restricted cases were stained with three lots of VENTANA Kappa/Lambda Dual ISH Cocktail, three production lots of VENTANA SILVER ISH BF Detection Kit, and three production lots of VENTANA MAGENTA ISH DIG Detection kit on three BenchMark ULTRA instrument systems. All slides were evaluated for restriction status. For lot-to-lot reproducibility, three slides from each tissue case were stained across three lots of the kit for a total of 78 slides.

The on-slide tonsil control is used to determine that the slide had all necessary reagents dispensed successfully and the assay performed as intended. The results are shown in Table 7. Precision results for each sample in the lot-to-lot study are presented in Table 8 and demonstrate that the mean ratios fall within the expected restriction category.

Table 7. Lot-to-lot Reagent Concordance

Case #	Run Result			Qualification Result	Total Concordant Observations / Total Observations
	1	2	3		
1	Lambda	Lambda	Lambda	Lambda	3/3
2	Kappa	Kappa	Kappa	Kappa	3/3
3	Lambda	Lambda	Lambda	Lambda	3/3
4	Lambda	Lambda	Lambda	Lambda	3/3
5	Kappa	Kappa	Kappa	Kappa	3/3
6	Lambda	Lambda	Lambda	Lambda	3/3
7	Lambda	Lambda	Lambda	Lambda	3/3
8	Kappa	Kappa	Kappa	Kappa	3/3
9	Lambda	Lambda	Lambda	Lambda	3/3
10	Lambda	Lambda	Lambda	Lambda	3/3
11	Kappa	Kappa	Kappa	Kappa	3/3
12	Kappa	Kappa	Kappa	Kappa	3/3
13	Kappa	Kappa	Kappa	Kappa	3/3
14	Lambda	Lambda	Lambda	Lambda	3/3
15	Kappa	Kappa	Kappa	Kappa	3/3
16	Kappa	Kappa	Kappa	Kappa	3/3
17	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
18	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
19	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
20	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
21	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
22	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
23	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
24	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
25	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3
26	Non- restricted	Non- restricted	Non- restricted	Non- restricted	3/3

Table 8. Precision results for lot-to-lot study by sample.

	Kappa/Lambda Status					Kappa/Lambda Derived Ratio				
Case #	N	Case-Level Mode Status	Kappa Restricted	Lambda Restricted	Non-Restricted	Mean	Median	Min, Max	SD	%CV
			N (%)							
1	3	Kappa Restricted	3 (100.0)	0	0	72.33	99.00	19.00, 99.00	46.19	63.85
2	3	Kappa Restricted	3 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
3	3	Kappa Restricted	3 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
4	3	Kappa Restricted	3 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
5	3	Kappa Restricted	3 (100.0)	0	0	45.67	19.00	19.00, 99.00	46.19	101.14

Case #	N	Case-Level Mode Status	Kappa/Lambda Status			Kappa/Lambda Derived Ratio				
			Kappa Restricted	Lambda Restricted	Non-Restricted	Mean	Median	Min, Max	SD	%CV
			N (%)							
6	3	Kappa Restricted	3 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
7	3	Kappa Restricted	3 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
8	3	Kappa Restricted	3 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
9	3	Lambda Restricted	0	3 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
10	3	Lambda Restricted	0	3 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
11	3	Lambda Restricted	0	3 (100.0)	0	0.05	0.05	0.05, 0.05	0.00	0.00
12	3	Lambda Restricted	0	3 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
13	3	Lambda Restricted	0	3 (100.0)	0	0.05	0.05	0.05, 0.05	0.00	0.00
14	3	Lambda Restricted	0	3 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
15	3	Lambda Restricted	0	3 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
16	3	Lambda Restricted	0	3 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
17	3	Non-Restricted	0	0	3 (100.0)	2.56	2.33	2.33, 3.00	0.38	15.06
18	3	Non-Restricted	0	0	3 (100.0)	0.78	0.67	0.67, 1.00	0.19	24.74
19	3	Non-Restricted	0	0	3 (100.0)	1.22	1.22	1.22, 1.22	0.00	0.00
20	3	Non-Restricted	0	0	3 (100.0)	1.62	1.50	1.50, 1.86	0.21	12.73
21	3	Non-Restricted	0	0	3 (100.0)	3.00	3.00	3.00, 3.00	0.00	0.00
22	3	Non-Restricted	0	0	3 (100.0)	2.02	1.86	1.86, 2.33	0.27	13.64
23	3	Non-Restricted	0	0	3 (100.0)	2.33	2.33	2.33, 2.33	0.00	0.00
24	3	Non-Restricted	0	0	3 (100.0)	2.33	2.33	2.33, 2.33	0.00	0.00
25	3	Non-Restricted	0	0	3 (100.0)	1.07	1.00	1.00, 1.22	0.13	11.94
26	3	Non-Restricted	0	0	3 (100.0)	1.15	1.22	1.00, 1.22	0.13	11.17

The study demonstrates lot-to-lot reproducibility of VENTANA K/L Probe Cocktail, VENTANA SILVER ISH BF Detection Kit, and VENTANA MAGENTA ISH DIG Detection Kit by meeting the acceptance criteria of a 95% lower bound confidence interval of 80%. All replicates for each of the 3 lots resulted in the same call.

Intra-run (day), Inter-run (day) and Inter-instrument Reproducibility

This study was conducted to compare tissue samples stained in a single ULTRA run (intra-run), to compare tissue samples stained over multiple non-consecutive days (inter-run), and to compare tissue samples stained with different ULTRA instruments (inter-instrument), to demonstrate that the assay shall produce concordant staining results in at least 90% of samples, with an OPA of at least 80%.

For this study, 26 cases representing a range of restriction statuses including 2 borderline cases with 1 each of Kappa and Lambda were stained with the VENTANA K/L Probe Cocktail on multiple ULTRAs and across multiple days. To evaluate for intra-run reproducibility, cases were stained in duplicate on all runs. To evaluate inter-run reproducibility, cases were stained across five days on the same ULTRA. To evaluate for inter-instrument reproducibility, cases were stained on three different ULTRAs. Stained slides were scored for restriction status, acceptable background, and acceptable morphology. Results show 100% concordance (Table 9) for detection of kappa and lambda expression for with-run, between day, and between instrument performance. Overall precision results for each sample are presented in Table 10 and demonstrate that the mean ratios fall within the expected restriction category.

Table 9. Results for repeatability and intermediate precision testing on the BenchMark ULTRA instrument.

Study	n/N	APA Kappa (%)	95% CI	APA Lambda (%)	95% CI	OPA (%)	95% CI
Within-Run Repeatability	178/178	100	96.6, 100	100	96.6, 100	100	97.9, 100.0
Between-Day Intermediate Precision	256/256	100	95.4, 100	100	95.3, 100	100	98.5, 100.0
Between-Instrument Intermediate Precision	156/156	100	92.6, 100	100	92.6, 100	100	97.6, 100.0

Table 10. Precision results by sample for intermediate precision study

	Kappa/Lambda Status					Kappa/Lambda Derived Ratio					
Case #	N	Case-Level Mode Status	Kappa Restricted	Lambda Restricted	Non-Restricted	N	Mean	Median	Min, Max	SD	%CV
			N (%)								
1	14	Kappa Restricted	14 (100.0)	0	0	14	30.43	19.00	19.00, 99.00	29.05	95.47
2	14	Kappa Restricted	14 (100.0)	0	0	14	30.43	19.00	19.00, 99.00	29.05	95.47
3	14	Kappa Restricted	14 (100.0)	0	0	14	24.71	19.00	19.00, 99.00	21.38	86.51
4	14	Kappa Restricted	14 (100.0)	0	0	14	10.43	9.00	9.00, 19.00	3.63	34.82
5	14	Kappa Restricted	14 (100.0)	0	0	14	10.43	9.00	9.00, 19.00	3.63	34.82
6	13	Kappa Restricted	13 (100.0)	0	0	13	49.77	19.00	19.00, 99.00	40.51	81.39

Case #	Kappa/Lambda Status					Kappa/Lambda Derived Ratio					
	N	Case-Level Mode Status	Kappa Restricted	Lambda Restricted	Non-Restricted	N	Mean	Median	Min, Max	SD	%CV
			N (%)								
7	14	Kappa Restricted	14 (100.0)	0	0	14	70.43	99.00	19.00, 99.00	39.78	56.48
8	14	Kappa Restricted	14 (100.0)	0	0	14	99.00	99.00	99.00, 99.00	0.00	0.00
9	14	Lambda Restricted	0	14 (100.0)	0	14	0.07	0.05	0.01, 0.11	0.04	54.65
10	14	Lambda Restricted	0	14 (100.0)	0	14	0.10	0.11	0.05, 0.11	0.02	20.68
11	14	Lambda Restricted	0	14 (100.0)	0	14	0.05	0.05	0.01, 0.05	0.01	22.92
12	14	Lambda Restricted	0	14 (100.0)	0	14	0.11	0.11	0.11, 0.11	0.00	0.00
13	14	Lambda Restricted	0	14 (100.0)	0	14	0.03	0.01	0.01, 0.05	0.02	83.60
14	13	Lambda Restricted	0	13 (100.0)	0	13	0.01	0.01	0.01, 0.01	0.00	0.00
15	14	Lambda Restricted	0	14 (100.0)	0	14	0.05	0.05	0.01, 0.05	0.02	33.17
16	13	Lambda Restricted	0	13 (100.0)	0	13	0.08	0.11	0.05, 0.11	0.03	36.09
17	14	Non-Restricted	0	0	14 (100.0)	14	1.62	1.50	1.50, 2.33	0.30	18.69
18	14	Non-Restricted	0	0	14 (100.0)	14	1.00	1.00	1.00, 1.00	0.00	0.00
19	14	Non-Restricted	0	0	14 (100.0)	14	1.00	1.00	1.00, 1.00	0.00	0.00
20	13	Non-Restricted	0	0	13 (100.0)	13	1.12	1.00	1.00, 1.50	0.22	19.66
21	14	Non-Restricted	0	0	14 (100.0)	14	3.00	3.00	3.00, 3.00	0.00	0.00
22	14	Non-Restricted	0	0	14 (100.0)	14	1.00	1.00	1.00, 1.00	0.00	0.00
23	14	Non-Restricted	0	0	14 (100.0)	14	2.13	2.33	1.50, 3.00	0.54	25.33
24	14	Non-Restricted	0	0	14 (100.0)	14	2.33	2.33	2.33, 2.33	0.00	0.00
25	14	Non-Restricted	0	0	14 (100.0)	14	2.21	2.33	1.50, 2.33	0.30	13.67
26	14	Non-Restricted	0	0	14 (100.0)	14	1.17	1.11	1.00, 1.50	0.20	17.41

All acceptance criteria were met for this study as established and produced high concordance. This data suggests the VENTANA K/L Probe Cocktail is appropriately reproducible on the BenchMark ULTRA across instruments and runs.

Inter- and Intra-reader Reproducibility

This study was designed to evaluate inter- and intra-reader reproducibility of restriction status scoring with the assay. For the study, 60 stained cases were scored for restriction status by three pathologists in two rounds, with each round being separated by a minimum two-week wash-out period. Cases represented a range of expression levels and restriction statuses, including 18 Kappa restricted, 18 Lambda restricted, 18 non-restricted, 3 Kappa borderline restricted, and 3 Lambda borderline restricted cases. Intra-reader performance was assessed through pooled round one and round two overall case concordance for each reader. Inter-reader performance was assessed through an overall pairwise comparison of round one scores for all three readers. The acceptance criteria for both studies were set at 90% concordance using 2 BenchMark ULTRA instruments. Results in Table 11 show that concordance was >90% for inter-reader and intra-reader precision for both Kappa restriction and Lambda restriction. These results demonstrate that the VENTANA K/L Probe Cocktail produces a staining pattern that can be reproducibly interpreted by a single reader and across multiple different readers. Reader precision results by sample are presented in Table 12.

Table 11. Results for reader precision testing.

Study	n/N	APA Kappa (%)	95% CI	APA Lambda (%)	95% CI	OPA (%)	95% CI
Within-Reader Precision	180/180	100	96.7, 100	100	96.6, 100	100	97.9, 100.0
Between-Reader Precision	352/360	96.4	91.5, 100	100	98.3, 100	97.8	94.4, 100.0

Table 12. Reader precision results by sample.

	Kappa/Lambda Status					Kappa/Lambda Derived Ratio				
Case #	N	Case-Level Mode Status	Kappa Restricted	Lambda Restricted	Non-Restricted	Mean	Median	Min, Max	SD	%CV
			N (%)							
1	6	Kappa Restricted	6 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
2	6	Kappa Restricted	6 (100.0)	0	0	59.00	59.00	19.00, 99.00	43.82	74.27
3	6	Kappa Restricted	6 (100.0)	0	0	85.67	99.00	19.00, 99.00	32.66	38.12
4	6	Kappa Restricted	6 (100.0)	0	0	85.67	99.00	19.00, 99.00	32.66	38.12
5	6	Kappa Restricted	6 (100.0)	0	0	59.00	59.00	19.00, 99.00	43.82	74.27
6	6	Kappa Restricted	6 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
7	6	Kappa Restricted	6 (100.0)	0	0	41.50	19.00	4.00, 99.00	44.92	108.23
8	6	Kappa Restricted	6 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
9	6	Kappa Restricted	6 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
10	6	Kappa Restricted	6 (100.0)	0	0	85.67	99.00	19.00, 99.00	32.66	38.12
11	6	Kappa Restricted	6 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00

Case #	Kappa/Lambda Status					Kappa/Lambda Derived Ratio				
	N	Case-Level Mode Status	Kappa Restricted	Lambda Restricted	Non-Restricted	Mean	Median	Min, Max	SD	%CV
			N (%)							
12	6	Kappa Restricted	6 (100.0)	0	0	57.33	59.00	9.00, 99.00	45.79	79.87
13	6	Kappa Restricted	6 (100.0)	0	0	45.67	19.00	19.00, 99.00	41.31	90.46
14	6	Kappa Restricted	6 (100.0)	0	0	8.72	7.33	4.00, 19.00	5.42	62.11
15	6	Kappa Restricted	6 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
16	6	Kappa Restricted	6 (100.0)	0	0	72.33	99.00	19.00, 99.00	41.31	57.11
17	6	Kappa Restricted	6 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
18	6	Kappa Restricted	6 (100.0)	0	0	99.00	99.00	99.00, 99.00	0.00	0.00
19	6	Lambda Restricted	0	6 (100.0)	0	0.02	0.01	0.01, 0.05	0.02	90.44
20	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
21	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
22	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
23	6	Lambda Restricted	0	6 (100.0)	0	0.02	0.01	0.01, 0.05	0.02	100.97
24	6	Lambda Restricted	0	6 (100.0)	0	0.02	0.01	0.01, 0.05	0.02	100.97
25	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
26	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
27	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
28	6	Lambda Restricted	0	6 (100.0)	0	0.04	0.05	0.01, 0.05	0.02	57.10
29	6	Lambda Restricted	0	6 (100.0)	0	0.02	0.01	0.01, 0.05	0.02	90.44
30	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
31	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
32	6	Lambda Restricted	0	6 (100.0)	0	0.02	0.01	0.01, 0.05	0.02	90.44
33	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
34	6	Lambda Restricted	0	6 (100.0)	0	0.02	0.01	0.01, 0.05	0.02	100.97
35	6	Lambda Restricted	0	6 (100.0)	0	0.10	0.08	0.01, 0.33	0.12	115.35

Case #	Kappa/Lambda Status					Kappa/Lambda Derived Ratio				
	N	Case-Level Mode Status	Kappa Restricted	Lambda Restricted	Non-Restricted	Mean	Median	Min, Max	SD	%CV
			N (%)							
36	6	Lambda Restricted	0	6 (100.0)	0	0.01	0.01	0.01, 0.01	0.00	0.00
37	6	Non-Restricted	0	0	6 (100.0)	1.88	1.54	1.00, 3.00	0.91	48.36
38	6	Non-Restricted	0	0	6 (100.0)	1.41	1.36	1.00, 1.86	0.39	28.08
39	6	Non-Restricted	0	0	6 (100.0)	1.36	1.22	1.00, 1.86	0.40	29.26
40	6	Non-Restricted	0	0	6 (100.0)	1.48	1.22	1.00, 2.33	0.51	34.54
41	6	Non-Restricted	0	0	6 (100.0)	1.36	1.22	1.00, 1.86	0.40	29.26
42	6	Non-Restricted	0	0	6 (100.0)	1.63	1.68	1.00, 2.33	0.49	29.81
43	6	Non-Restricted	0	0	6 (100.0)	1.32	1.11	1.00, 1.86	0.42	31.96
44	6	Non-Restricted	0	0	6 (100.0)	1.30	1.22	1.00, 1.86	0.33	25.33
45	6	Non-Restricted	0	0	6 (100.0)	1.53	1.50	1.00, 2.33	0.51	33.49
46	6	Non-Restricted	0	0	6 (100.0)	1.45	1.50	1.00, 1.86	0.39	26.51
47	6	Non-Restricted	0	0	6 (100.0)	1.79	1.92	1.00, 2.33	0.62	34.64
48	6	Non-Restricted	0	0	6 (100.0)	1.23	1.00	1.00, 1.86	0.37	30.02
49	6	Non-Restricted	1 (16.7)	0	5 (83.3)	2.89	1.86	0.82, 9.00	3.03	104.80
50	6	Non-Restricted	0	0	6 (100.0)	1.35	1.36	1.00, 1.86	0.34	24.93
51	6	Non-Restricted	0	0	6 (100.0)	1.41	1.36	1.00, 1.86	0.39	28.08
52	6	Non-Restricted	0	0	6 (100.0)	1.61	1.86	1.00, 1.86	0.39	24.35
53	6	Non-Restricted	0	0	6 (100.0)	1.50	1.54	1.00, 1.86	0.40	26.40
54	6	Non-Restricted	0	0	6 (100.0)	1.36	1.22	1.00, 1.86	0.40	29.26
55	6	Non-Restricted	0	0	6 (100.0)	1.78	1.68	1.00, 3.00	0.79	44.16
56	6	Non-Restricted	0	0	6 (100.0)	1.54	1.54	1.00, 2.33	0.55	35.62
57	6	Non-Restricted	1 (16.7)	0	5 (83.3)	3.48	2.33	1.86, 9.00	2.73	78.55
58	6	Non-Restricted	0	0	6 (100.0)	1.00	1.00	0.67, 1.50	0.28	28.15
59	6	Non-Restricted	0	0	6 (100.0)	1.51	1.50	1.00, 2.33	0.45	29.94

Case #	Kappa/Lambda Status					Kappa/Lambda Derived Ratio				
	N	Case-Level Mode Status	Kappa Restricted	Lambda Restricted	Non-Restricted	Mean	Median	Min, Max	SD	%CV
			N (%)							
60	6	Non-Restricted	0	0	6 (100.0)	1.02	1.00	0.82, 1.50	0.25	24.46

2. Analytical Sensitivity and Specificity

This study was designed to evaluate sensitivity, specificity, and first pass rate of this probe and associated detection kits on the BenchMark ULTRA instrument. For sensitivity, 48 cases with known restriction by flow cytometry were stained with VENTANA K/L Probe Cocktail and scored for restriction (Table 13). Sensitivity was assessed by evaluation of concordance of the VENTANA K/L Probe Cocktail restriction calls to those by flow cytometry.

For the VENTANA K/L Probe Cocktail assay, specificity was assessed by staining a large panel of tissues and evaluating the stained slides for appropriate K/L signal. The tested panel of tissues included the cases stained for sensitivity testing, a tour of tumor tissue array, a T-cell tissue array, and a tour of body array (including a subset of normal tissue cases). Staining pattern with neoplastic tissue is shown in Table 14. Passing signal was defined as appropriate cytoplasmic staining in B-cells or plasma cells and a lack of signal in all other cell types. ISH Negative Control-stained slides were used to assess detection kit background. The assay was tested in a tour of tumor/tour of body study that produced acceptable results. For normal tissue, staining was seen in lymph node (15/15 cases), tonsil (5/5 cases) and bone marrow (5/5 cases). For neoplastic tissues, staining was only seen in B-cell lymphomas. These results suggest that the assay will detect B-cell neoplasms and normal B-cell reactive processes.

Table 13. Normal tissue cases stained with KL Probe Cocktail. Tissues marked with an asterisk exhibited scattered normal B-lymphocyte staining.

Tissue Type	Positive Cases / Total Cases	Tissue Type	Positive Cases / Total Cases
Brain	0/1	Bone marrow	5/5
Cerebrum	0/3	Lung*	0/5
Cerebellum	0/4	Heart	0/4
Adrenal gland*	0/4	Esophagus*	0/4
Ovary*	0/4	Stomach*	0/4
Pancreas*	0/4	Small intestine*	0/4
Lymph node	15/15	Colon*	0/6
Parathyroid gland	0/3	Liver*	0/4
Pituitary gland	0/3	Salivary gland*	0/4
Testis*	0/4	Kidney*	0/5
Thyroid	0/4	Prostate*	0/4
Breast*	0/5	Cervix	0/6
Spleen*	0/4	Skin	0/4
Tonsil	5/5	Mesothelial	0/4
Skeletal muscle*	0/3	Rectum	0/1
Peripheral nerve	0/4	Placenta	0/3
Bladder	0/4	Uterus*	0/4
Thymus*	0/4		

Table 14. Neoplastic tissue cases stained with K/L Probe Cocktail. Includes all tour of tumor, t-cell array, and cases with flow data.

Neoplastic Tissue Type	Positive Cases / Total Cases
Astrocytoma (Brain)	0/1
Meningioma, (Brain)	0/3
Adenocarcinoma (Head and neck)	0/1
Melanoma (Nasal cavity)	0/1
Nasopharyngeal carcinoma, (Nasopharynx)	0/1
Squamous cell carcinoma (Tongue)	0/1
Adenoma (Adrenal gland)	0/1
Adrenocortical carcinoma (Adrenal gland)	0/1
Colonic signet ring cell carcinoma, metastatic (Ovary)	0/1
Adenocarcinoma (Ovary)	0/2
Granulosa cell tumor (Ovary)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Adenoma (Thyroid)	0/3
Follicular carcinoma (Thyroid)	0/1
Follicular papillary adenocarcinoma (Thyroid)	0/1
Invasive ductal carcinoma (Breast)	0/3
Fibroadenoma (Breast)	0/2
Osteosarcoma (Bone)	0/1
Chondrosarcoma (Bone)	0/1
Adenocarcinoma (Lung)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Gastrointestinal carcinoma, metastatic (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/2
Adenocarcinoma (Stomach)	0/3
Adenoma (Small intestine)	0/1
Adenocarcinoma (Small intestine)	0/1
Adenoma (Colon)	0/1
Adenocarcinoma (Colon)	0/3
Colonic adenocarcinoma, metastatic (Liver)	0/1
Hepatocellular carcinoma (Liver)	0/4
Pleomorphic adenoma (Parotid salivary gland)	0/1
Adenoid cystic carcinoma (Salivary gland)	0/1
Clear cell carcinoma (Kidney)	0/2
Adenocarcinoma (Prostate)	0/2
Adenocarcinoma (Uterus)	0/2
Squamous cell carcinoma (Cervix)	0/2
Squamous cell carcinoma (Skin)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/2
T-Cell lymphoblastic lymphoma	0/1
T cell lymphoma, Mycosis fungoides	0/1
T cell lymphoma, Lennert lymphoma	0/2
T cell lymphoma, enteropathy-associated	0/5
T cell lymphoma, angioimmunoblastic	0/6
T cell lymphoma, NOS	0/20
NK/T-cell lymphoma, nasal type	0/5
NK/T-cell lymphoma (Testis)	0/1

Neoplastic Tissue Type	Positive Cases / Total Cases
Hodgkin lymphoma	0/1
B-cell lymphoma, NOS	1/1
Follicular lymphoma	7/7
Mantle cell lymphoma	4/4
Diffuse large B-cell lymphoma	11/11
Plasma cell neoplasms	4/4
Marginal zone lymphoma	3/3
Chronic lymphocytic leukemia/small lymphocytic lymphoma	5/5
Urothelial carcinoma (Bladder)	0/2
Squamous cell carcinoma, metastatic (Lymph node)	0/1
Adenocarcinoma (Rectum)	0/3

Robustness of Staining

a. Tissue Thickness

The Pre-analytic Study characterizes the effect of cut slide tissue thickness and pre-analytic conditions to select while using VENTANA K/L Probe Cocktail. This study will first characterize tissue thickness, samples were sectioned between 2 to 8 microns. Cases were processed in duplicate for each case (2, 4, 6, 8 microns) for a total of 4 pairs per case, and each pair was stained with the VENTANA K/L Probe Cocktail. Stained slides were scored for restriction. Cases were selected to include Kappa restricted lymphomas, Lambda restricted lymphomas and tonsil. All 3 cases passed at all 4 thicknesses. The recommended thickness is 4 microns.

b. Fixation

This study also characterizes fixation, decalcification solutions and times. Tonsil tissue was fixed in various fixation solutions for specific time points (0.5 hr, 1 hr, 6 hr, 12 hr, 24 hr and 48 hr). For characterizing decalcification, tissue was decalcified in various solutions for specific time points (0.5 hr, 1 hr, 6 hr, 12 hr, 24 hr and 48 hr) followed by being fixed in one of two fixatives, 10% NBF or Zinc Formalin, for 24 hours. Pre-analytic staining for tissue thickness and fixation/decalcification runs were conducted on the BenchMark ULTRA instruments. Acceptable staining was observed using multiple fixation solutions. The recommended fixation was with 10% NBF for 6 – 72 hours.

3. Stability

Real Time and Ship Stress Reagent Stability Interim Report

The objective of this study was to verify the shelf-life and in-use stability and shipping category of VENTANA K/L Probe Cocktail, the VENTANA Silver ISH BF Detection Kit, the VENTANA Magenta ISH DIG Detection Kit, and ISH TSA Ancillary Kit. Stability was assessed by evaluating the assay's staining performance on kappa restricted lymphomas, lambda restricted lymphomas, and tonsil (non-restricted) tissues. Data from this study will be used to support product dating and shipping category of VENTANA K/L Probe Cocktail. All cases were required to be 100% concordant to Day 0 slides. The final goal of this study was 26 months testing to support product dating of 24 months. The following table (Table 15) summarizes the real time stability testing results for three lots of VENTANA K/L Probe Cocktail from Day 0 to Month 7. Kits were stored at 2 – 8°C at the intended storage (IS) and then tested under hot shipping conditions (37 ± 2°C) or 3 freeze/thaw conditions (F/T). Interim results support stability at 7 months.

Table 15. Real-Time and Ship Stress Stability Results

Time Point (Month)	Results (Pass/Fail/Invalid)								
	Lot 1			Lot 2			Lot 3		
	IS	Hot Ship	F/T cycles	IS	Hot Ship	F/T cycles	IS	Hot Ship	F/T cycles
Day 0	Pass	N/A		Pass	N/A		Pass	N/A	
Month 7	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass

Cut Slide Stability

The objective of this study was to assess the stability of the VENTANA K/L Probe Cocktail targets in specimen cut slides. This study is used to support cut slide stability when slides are stored at 5°C for through 12 months. Stability was evaluated in triplicate at multiple time points at 5°C ± 3°C and at 30°C ± 5°C using one reagent lot on lymphoma and tonsil tissue. In order to pass for a stability time point, 100% of slides at that time point must have a restriction status concordant to the corresponding day 0 time point restriction status.

The study was conducted out to 397 days with slides stored at 5°C ± 3°C or 30°C ± 5°C with multiple tissue types. The results demonstrated 100% concordance. This data suggest that the stability of test tissue slides is at least 12 months at 5°C and 30°C temperature based on staining and kappa/lambda restriction compared to Day 0.

B Clinical Performance Data:

1. Comparison Study:

Clinical performance of the assay was assessed using final pathology diagnosis associated with the same patient in addition to a study comparing the clinical performance of VENTANA K/L Probe Cocktail as used on the BenchMark ULTRA instrument in determining immunoglobulin (Ig) light-chain restriction status in human bone marrow and lymphoid tissues as an aid in the identification of B-cell lymphomas and plasma cell neoplasms, where restriction status is defined by the ratio of Kappa (K) mRNA to Lambda (L) mRNA (K:L); where if the ratio K:L is ≥ 4:1, the case is K-restricted; if the K:L ratio is ≤ 1:2, the case is L-restricted.

The study included personnel at 3 clinical/ anatomical pathology laboratories (study sites) who performed a systematic search of their laboratory records to identify approximately 200 bone marrow or lymphoid patient tissue samples (per site) that were associated with a diagnostic workup including K:L testing by flow cytometry to aid in the identification of B-cell lymphoma or plasma cell neoplasms. The clinical sites stained 869 of these cases with VENTANA K/L Probe Cocktail on a BenchMark ULTRA instrument, and 811 of the 869 stained cases had acceptable H&E and were evaluated for K/L staining by the screening pathologist. A total of 742 cases that met enrollment criteria were enrolled into the study and up to 4 pathologists from the sites each evaluated all enrolled cases. Performance of the VENTANA K/L Probe Cocktail was assessed as percent agreement between the ISH assay and historical flow results for each restriction category (kappa restricted, lambda restricted, or non-restricted) and as an overall percent agreement (OPA) for all cases.

The stained slides for evaluable cases stained with the VENTANA K/L Probe Cocktail were independently interpreted for restriction status by 4 study pathologists across the 3 study sites, and the pathologists' assessments for each case were compared to the historical flow derived K/L restriction status and final pathology diagnosis associated with the same patient to assess the performance of the assay. The rate of invalid tests/indeterminates was 2.4% as determined by this study.

The co-primary endpoints in this study were the 95% CI lower bounds associated with the KRPA, LRPA, and NRPA point estimates for K/L restriction status agreement between the VENTANA K/L Probe Cocktail reader observations and the historical flow result associated with the same patient, with the flow result as the reference. Reader observations indicating co-expression of K and L mRNA were excluded from the primary analysis. As shown in Table 16 below, the resulting KRPA, LRPA, and NRPA point estimates, pooled across all evaluable observations, were 90.9% (95% CI: 87.9, 93.8), 94.8% (95% CI: 92.0, 97.3), and 95.2% (95% CI: 93.3, 96.8) (need to update results and table below) respectively, satisfying the study requirement for a minimum 95% CI lower bound of at least 85% for each co-primary endpoint. Although the study was designed for analysis of the combined co-primary endpoint, a separate analysis was also performed for plasma cell neoplasms and B-cell, separately. Results indicate lower performance with some B-cell lymphomas, particularly follicular lymphomas. However, in some cases, discordance may be due to the inability of flow cytometry to select regions of interest, as is recommended with the Kappa and Lambda Dual ISH Assay. Higher discordance was also observed with samples around the kappa and lambda cutoffs, which may be due in some cases to non-specific detection of IGLL5.

Table 16. Restriction status agreement between Ventana K/L Probe Cocktail Assay and from cytometry for all readers pooled.

Restriction Category	Agreement % (n/N)	95% CI*
Kappa Restricted	90.9% (836/920)	87.9% – 93.8%
Lambda Restricted	94.8% (746/787)	92.0% – 97.3%
Non-restricted	95.2% (810/851)	93.3% – 96.8%
Overall	93.5% (2392/2558)	92.0% – 95.0%

* Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates with stratification by the 6 applicable tissue-type/flow status enrollment bins used during case screening for the All Evaluable Cases population.
** The prevalence-weighted OPA was calculated using the co-primary point estimates for KRPA, LRPA, and NRPA and the prevalence of K-, L-, and non-restricted cases observed in Phase 1; the weighted OPA= (Kappa restriction prevalence x KRPA) + (Lambda restriction prevalence x LRPA) + (Non-restriction Prevalence x NRPA). Therefore, n/N is not shown.

VI Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

VII Identified Risks and Mitigations:

Risks to Health	Mitigation Measures
False positive or false negative results, or failure to produce results.	Certain design verification and validation, including certain studies and risk mitigation analysis. Certain labeling information, including limitations, device descriptions, methodology and protocols, and performance information.
"Incorrect interpretation of results by the user or physician"	Certain design verification and validation, including certain studies and risk mitigation analysis. Certain labeling information, including limitations, device descriptions, methodology and protocols, and performance information.

VIII Benefit/Risk Assessment:

A. Summary of the Assessment of Benefit:

The probable benefit of this device is the identification of Kappa restriction and Lambda restriction in B cell lymphomas and plasma cell neoplasms, to aid in the diagnosis of these disorders. In addition, this kit meets an unmet need, allowing for the use of available FFPE tissue, when fresh, live cells required for flow cytometry are not available or sufficient.

In summary, this clinical method comparison study shows that the performance of this device is clinically acceptable for both B-cell and plasma cell claims, and shows probable benefit for the indications sought, with the addition of specific labeling mentioned above. Please refer to the method comparison study above.

B. Summary of the Assessment of Risk:

There is probable risk associated with the use of this device, mainly due to 1) false negatives, false positives, and failure to provide a result and 2) incorrect interpretation of test results by the user. Erroneous device results could adversely impact the clinical diagnosis of B cell neoplasms or plasma cell neoplasms. False positive results could result in improper medical management of patients, which represent a significant clinical risk. A false negative result may result in a missed diagnosis of B cell neoplasms or plasma cell neoplasms. even though this device is intended only as an aid in diagnosis and not a standalone test and is intended to be used alongside with other clinico-pathological factors. Failure to produce results or an incorrect interpretation of enrichment results by the lab can result similar consequences.

C. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

D. Summary of the Assessment of Benefit-Risk:

The probable benefit of the device was demonstrated by a high percentage KRPA, LRPA and NRPA, compared to flow cytometry, which supports the probable benefits of this device for the indications sought as an aid in the diagnosis of B cell lymphomas and plasma cell neoplasms. The risks of false results, failure to provide results, or incorrect interpretation of results are

mitigated by the fact that this device is intended only as an aid in diagnosis and not a standalone test and is intended to be used alongside other clinico-pathological factors. Risks are further mitigated by various limitations in the labeling, as well as device verification and validation studies, which help to ensure the performance of the device.

The limitations of the device performance were carefully studied in different subgroups of cancers, to more clearly understand device performance. In addition, the performance of the device was supported by studies such as the limit of detection and precision, which inform users of the limitations of this device. A special control regarding risk mitigation strategies can also help reduce the potential negative consequences of false results. In addition, special user training instructions reduces the risk of miscalls particularly around staining in follicular lymphomas and some specimens that contain non-specific staining with IGLL5. Therefore, while general controls are not sufficient to address the risks of this device, in light of the special controls, the probable benefits of this device outweigh the probable risks of this device.

IX Conclusion:

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code: SDP
Device Type: Hematolymphoid neoplasm immunoglobulin mRNA in situ hybridization detection device
Class: II
Regulation: 21 CFR 864.1861