EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
EasySep Human Bone Marrow CD138 Positive Selection Kit
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN220090

B Applicant

STEMCELL Technologies Canada Inc.

C Proprietary and Established Names

EasySep™ Human Bone Marrow CD138 Positive Selection Kit

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QYO	Hematopoietic cell enrichment kit	21 CFR 866.6120	Immunology

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for EasySep Human Bone Marrow CD138 Positive Selection

B Measurand:

CD138 (Syndecan-1)

C Type of Test:

Tool for selection of human bone marrow CD138+ plasma cells

III Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

EasySep Human Bone Marrow CD138 Positive Selection Kit is an in vitro diagnostic device intended to enrich CD138+ cells from bone marrow collected from patients diagnosed with multiple myeloma. The CD138+ cells are enriched by immunomagnetic positive selection for use in validated downstream assays. The end-user is responsible for validation of this kit for use with the assay. For in vitro diagnostic use by laboratory professionals.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For in vitro diagnostic use

D Special Instrument Requirements:

Not applicable; The EasySep Human Bone Marrow CD138 Positive Selection Kit was validated for manual use.

IV Device/System Characteristics:

A Device Description:

The EasySep CD138 Human Bone Marrow CD138 Positive Selection Kit contains reagents to enrich CD138 positive (CD138+) human bone marrow cells. The kit contains reagents for up to 60 mL of bone marrow purifications. The kit contents are listed in Table 1. Materials required but not provided are shown in Table 2.

Table 1. EasySep Human Bone Marrow CD138 Positive Selection Kit Components

Component	Packaging Configuration	Storage	Content
EasySep Human Bone Marrow CD138 Positive Selection Cocktail (300-0394)	3 x 1 mL vials	Store at 2 - 8°C. Do not freeze.	A combination of monoclonal antibodies in Dulbecco's Phosphate Buffered Saline. Includes an Fc receptor blocking antibody and Recombinant Human Albumin stabilizer.
EasySep Dextran RapidSpheres 50105 (300-0400)	3 x 1 mL vials	Store at 2 - 8°C. Do not freeze.	A suspension of magnetic particles in water.

Table 2. Materials required but not provided (Sold separately)

Component	Configuration	Storage	Content
EasySep Red Blood Cell Lysis Buffer, 10x Concentrate (100-0749)	1 x 20 mL Bottle	Store at 15 - 25°C.	A 10x concentrated red blood cell lysis reagent.
EasySep Buffer (100-0780)	1 x 1 L	Store at 2 - 8°C. Do not freeze.	A Dulbecco's Phosphate Buffered Saline based cell separation buffer.
"The Big Easy" EasySep Magnet (18001)	1	Ambient temperature	Immunomagnetic column-free magnet.

B Principle of Operation

The EasySep Human Bone Marrow CD138 Positive Selection Kit is designed to enrich plasma cells expressing the CD138 surface antigen from fresh human whole bone marrow aspirates. Plasma cells are targeted with antibody complexes that crosslink CD138+ cells to dextran-coated magnetic particles. When the bone marrow specimen is placed into an EasySep magnet, CD138+ cells linked to magnetic particles are retained by the magnetic field while CD138- cells remain in suspension. This allows the CD138 negative (CD138-) cell fraction to be removed by pouring off the suspension containing unlabeled, unbound cells. Enriched CD138+ cells are collected by the removal of the sample from the magnet and can then be used in downstream applications.

V Standards/Guidance Documents Referenced:

CLSI Guideline EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures

CLSI Guideline EP07-A2: Interference Testing in Clinical Chemistry

CLSI Guideline EP25-A Evaluation of Stability of *In Vitro* Diagnostic Reagents

VI Performance Characteristics:

A Analytical Performance:

1. Reproducibility:

a) Reproducibility with Contrived Samples

A study was performed to demonstrate the reproducibility of the enrichment process. Due to challenges with having adequate sample for three sites, a study was performed using contrived samples generated by spiking a multiple myeloma cell line into healthy donor whole blood each day at 3 CD138+ levels based on flow cytometry events (low, medium, and high representing <3%, 3-15%, and >15%, respectively) to create 16 panel members. More panel members were studied at the low and medium levels (6 versus 4 for the high level) to demonstrate enrichment at the more challenging levels. Reproducibility was evaluated at three sites by two operators at each site for 8 non-consecutive days. Each of the three independent sites received six aliquots (from 16 panel members) from the same panel members. Each operator performed enrichments in duplicate using three different reagent lots on each panel member. After the enrichments were completed, all samples were given to a single operator to perform flow cytometry of the enriched and unenriched samples to measure CD138+ cell purity. Two different CD138 frequency contrived specimens were prepared for enrichments on each day.

Table 3 shows a statistical summary of the overall, site-to-site, lot-to-lot variability, and fold-enrichment from the high, medium, and low CD138+ panel members. Enrichment increased a mean of 85.7-fold in the low CD138+ initial frequency samples, 9.3-fold in the CD138+ medium frequency samples, and 3.8-fold in the high starting samples.

Table 3. EasySep CD138 Reproducibility Study Variability and Fold Enrichment Summary

CD138+ Initial Frequency	N	Repeatability CV (%)	Between Site CV (%)	Between Lot CV (%)	Reproducibility CV (%)	Fold Enrichment
		Mean (Min. - Max)				
High (> 15%)	4	0.7 (0.5 - 0.9)	4.0 (2.8 - 5.9)	0.9 (0.5 - 1.6)	4.0 (2.8 - 6.0)	3.8 (2.6 - 5.5)
Med (3 - 15%)	6	1.6 (0.8 - 3.0)	4.2 (2.1 - 6.8)	2.3 (1.0 - 3.9)	4.5 (2.2. - 7.0)	9.3 (5.0 - 15.4)
Low (< 3%)	6	5.4 (3.5 - 8.9)	9.7 (5.5 - 13.8)	8.6 (4.5 - 14.2)	11.9 (8.2 - 18.3)	85.7 (20.2 - 206.0)

Table 4 shows the mean plus minimum and maximum CD138+ purity for unenriched and enriched samples from all panel members across the three study sites. Low CD138+ panel members had mean CD138+ cell purities ranging from 0.7% - 1.5% before enrichment and increased to mean CD138+ cell purities of 56.2% - 79.6% after EasySep enrichment. Medium CD138+ panel members had mean CD138+ cell purities ranging from 7.0% - 12.3% before enrichment and increased to mean CD138+ cell purities of 85.5% - 92.6% after EasySep enrichment. High CD138+ panel members had mean CD138+ cell purities ranging from 17.9% - 22.8% before enrichment and increased to mean CD138+ cell purities of 88.5% - 94.0% after EasySep enrichment.

Table 4. Unenriched and Enriched CD138+ Cell Purity for Low, Medium, and High CD138+ Panel Members as Determined by FACS Analysis by All Three Study Sites

Target Freq	Panel Member	Unenriched Purity			Enriched Purity		
		Mean	Min	Max	Mean	Min	Max
Low	1	1.5	0.8	2	56.6	43.1	74.9
	5	1.3	0.7	2	73	62.3	86.7
	8	0.7	0.6	0.8	56.2	43.8	66.5
	12	0.7	0.4	0.9	76.9	66.4	89.3
	14	1.2	0.6	1.8	79.6	71.4	91.2
	15	0.8	0.4	1.2	74.9	57.9	82.8
Med	2	9.4	7.2	11.3	89.8	79.6	95.3
	3	9.2	7	12.5	88.9	84.3	93
	7	7.3	5.3	9.9	86.3	81.5	90.4
	10	7	5.5	8.3	86.4	75.3	93.2
	13	12.3	11.1	14.3	92.6	89.7	95.4
	16	10.4	6.2	14.6	85.5	78.7	90.4
High	4	19	16.4	21.3	94	89.9	96.8
	6	22.8	19.3	26.6	92.3	87.8	96.4
	9	18	17	18.6	88.5	82.9	95.2
	11	17.9	14.3	20	93.2	90.3	96.3

Separate analyses of variance were performed within each of the three sites and the three kit lots. Table 5 shows analysis results by kit lot and panel member, with precision estimates for repeatability and within lot/between sites. Similarly, Table 6 shows analysis results by site and panel member, with precision estimates for repeatability and within site/between lots. In both sets of analyses, all medium and high CD138+ panel members within site and lot had CV < 15%. Similarly, all low CD138+ panel members within site and lot had CV < 35%, which met the acceptance criteria due to the expected higher variability for low CD138+ starting samples. Thus, the variability is well-controlled within lot (across site) and within site (across lot).

Table 5. Precision Estimates by Kit Lot

Kit Lot	Panel Member	Mean Start Purity*	Mean Enriched Purity**	Repeatability		Within Lot	
				SD	CV (%)	SD	CV (%)
1	8	0.66	60.17	2.13	3.5	5.01	8.3
	12	0.71	72.48	1.21	1.7	8.39	11.6
	15	0.82	68.15	4.93	7.2	6.23	9.1
	14	1.19	77.56	1.71	2.2	4.56	5.9
	5	1.26	75.47	0.38	0.5	9.86	13.1
	1	1.47	55.66	7.13	12.8	7.18	12.9
	10	6.96	87.05	3.4	3.9	5.22	6
	7	7.27	87.26	0.33	0.4	1.17	1.3
	3	9.19	88.02	1.86	2.1	2.42	2.8
	2	9.44	88.91	0.65	0.7	7.47	8.4
	16	10.44	85.49	1.38	1.6	4.93	5.8
	13	12.34	92.23	0.71	0.8	2.1	2.3
	11	17.9	93.32	0.14	0.2	2.49	2.7
	9	18.01	87.11	1.13	1.3	5.02	5.8
	4	18.99	93.66	0.35	0.4	2.94	3.1
	6	22.84	92	0.45	0.5	3.99	4.3
2	8	0.66	55.67	1.68	3	9.79	17.6
	12	0.71	80.84	1.96	2.4	5.2	6.4
	15	0.82	79.59	1.14	1.4	1.96	2.5
	14	1.19	84.84	2.08	2.4	6.17	7.3
	5	1.26	72.02	2.72	3.8	6.6	9.2
	1	1.47	64.12	2.93	4.6	9.24	14.4
	10	6.96	88.4	2.54	2.9	4.74	5.4
	7	7.27	87.78	0.56	0.6	1.93	2.2
	3	9.19	90.19	0.85	0.9	2.19	2.4
	2	9.44	91.62	0.84	0.9	4.65	5.1
	16	10.44	84.9	0.96	1.1	3.91	4.6
	13	12.34	93.26	1.01	1.1	2.1	2.3
	11	17.9	93.36	0.78	0.8	2.54	2.7
	9	18.01	88.89	0.32	0.4	5.65	6.4
	4	18.99	94.27	0.72	0.8	3.12	3.3
	6	22.84	92.08	0.38	0.4	3.96	4.3
3	8	0.66	52.7	3.68	7	6.54	12.4
	12	0.71	77.45	3.58	4.6	7.67	9.9
	15	0.82	77.05	1.55	2	3.22	4.2
	14	1.19	76.55	2.44	3.2	4.08	5.3
	5	1.26	71.49	1.95	2.7	6.85	9.6
	1	1.47	50.17	1.73	3.5	6.66	13.3
	10	6.96	83.71	1.16	1.4	6.84	8.2
	7	7.27	83.86	0.93	1.1	2.56	3.1
	3	9.19	88.37	0.38	0.4	3.2	3.6
	2	9.44	88.72	0.69	0.8	5.9	6.7
	16	10.44	86.19	1.18	1.4	2.4	2.8
	13	12.34	92.18	0.81	0.9	1.68	1.8
	11	17.9	92.97	0.26	0.3	2.7	2.9
	9	18.01	89.43	0.77	0.9	4.97	5.6

Kit Lot	Panel Member	Mean Start Purity*	Mean Enriched Purity**	Repeatability		Within Lot	
				SD	CV (%)	SD	CV (%)
	4	18.99	94.11	0.39	0.4	2.84	3
	6	22.84	92.88	0.86	0.9	2.81	3

Start purity = unenriched CD138+ cell purity

*Starting purity for the same sample is averaged across differing measurements from the three sites.

**Mean enriched purity is the average purity of enriched samples across three study sites.

Table 6. Precision Estimates by Site

Site	Panel Member	Start Purity*	Mean Enriched Purity**	Repeatability		Within Site	
				SD	CV (%)	SD	CV (%)
1	8	0.57	60.75	2.69	4.4	3.81	6.3
	12	0.79	84.73	2.98	3.5	3.13	3.7
	15	0.92	76.93	0.72	0.9	2.73	3.5
	14	1.19	76.97	2.55	3.3	3.5	4.5
	5	0.65	81.18	1.08	1.3	4.6	5.7
	1	1.58	64.33	6.66	10.4	10.24	15.9
	10	7.11	87.67	0.43	0.5	2.2	2.5
	7	6.59	85.89	0.71	0.8	2.2	2.6
	3	8.06	89.44	0.94	1.1	1.6	1.8
	2	9.83	93.35	0.49	0.5	0.86	0.9
	16	10.52	85.13	0.59	0.7	1.12	1.3
	13	11.13	92.7	0.47	0.5	0.62	0.7
	11	19.39	92.4	0.14	0.1	0.35	0.4
	9	17.03	86.78	0.9	1	1.22	1.4
	4	19.26	96.03	0.34	0.4	0.48	0.5
	6	22.61	92.49	0.35	0.4	0.73	0.8
2	8	0.6	50.2	2.97	5.9	5.51	11
	12	0.4	73.25	2.41	3.3	6.6	9
	15	0.4	75.6	1.2	1.6	7.93	10.5
	14	0.6	77.93	0.88	1.1	6.25	8
	5	1.1	71.23	0.85	1.2	2.31	3.2
	1	0.8	54.1	2.59	4.8	5.16	9.5
	10	5.5	80.72	3.9	4.8	4.91	6.1
	7	5.3	84.77	0.26	0.3	2.77	3.3
	3	7	86.23	0.52	0.6	1.65	1.9
	2	7.2	82.85	0.78	0.9	3.15	3.8
	16	6.2	82.32	1.85	2.3	3.08	3.7
	13	11.6	90.63	1.23	1.4	1.23	1.4
	11	14.3	91.18	0.8	0.9	0.8	0.9
	9	18.6	84.37	0.31	0.4	1.37	1.6
	4	16.4	90.63	0.82	0.9	0.82	0.9
	6	19.3	88.68	0.89	1	1.27	1.4
3	8	0.82	57.58	2.19	3.8	8.56	14.9
	12	0.94	72.78	1.86	2.6	4.31	5.9
	15	1.15	72.25	5.1	7.1	8.58	11.9
	14	1.79	84.05	2.43	2.9	6.43	7.7
	5	2.04	66.57	3.08	4.6	3.08	4.6
	1	2.03	51.52	3.38	6.6	7.76	15.1
	10	8.28	90.77	2	2.2	2.36	2.6
	7	9.93	88.23	0.85	1	1.83	2.1

Site	Panel Member	Start Purity*	Mean Enriched Purity**	Repeatability		Within Site	
				SD	CV (%)	SD	CV (%)
	3	12.5	90.9	1.78	2	1.78	2
	2	11.3	93.05	0.87	0.9	1.53	1.6
	16	14.6	89.13	0.67	0.7	0.78	0.9
	13	14.3	94.33	0.67	0.7	0.93	1
	11	20	96.08	0.2	0.2	0.2	0.2
	9	18.4	94.28	1.03	1.1	1.59	1.7
	4	21.3	95.37	0.1	0.1	0.37	0.4
	6	26.6	95.78	0.41	0.4	0.44	0.5

Start purity = unenriched CD138+ cell purity

*Starting purity for the same sample is averaged across all three sites.

**Mean enriched purity is the average purity of enriched samples across three kit lots.

The CD138 EasySep Reproducibility study showed the EasySep Human Bone Marrow CD138 Positive Selection Kit produced consistent enrichment when starting with a specimen of given volume and initial CD138+ cell frequency across three independent laboratories and three separately manufactured kit lots. The precision statistical analysis of all 16 panel members showed that the CD138 cell enrichment performance across three independent laboratories using three independent kit lots gave fold enrichment and overall variability passing the acceptance criteria, demonstrating reproducibility across sites and kit lots.

b) Reproducibility with Clinical Specimens

A study was performed to demonstrate the reproducibility of the enrichment process using a validated FISH assay. Three (3) lots of the EasySep Human Bone Marrow CD138 Positive Selection Kit were tested on multiple myeloma (MM) patient bone marrow aspirates (BMA) in duplicate for performance in downstream FISH assays (Table 7). A total of 9 clinical MM BMA were tested, where each specimen was split in half to test enrichment with two different EasySep Human Bone Marrow CD138 Positive Selection Kit lots. The percentage of abnormal nuclei were analyzed using 5 probes which detect common MM chromosomal abnormalities, including the t(11;14) translocation (CCND1/IGH XT), chromosomes 5, 9, and 15 aneusomies (D5S23, D5S72, CEP 9, CEP 15) and 13q14 deletion.

Table 7. Lot-to-Lot Combinations

Specimen	Lot	Specimen	Lot
MM Specimen 1	Lot 1	MM Specimen 6	Lot 2
	Lot 2		Lot 3
MM Specimen 2	Lot 1	MM Specimen 7	Lot 1
	Lot 3		Lot 2
MM Specimen 3	Lot 2	MM Specimen 8	Lot 1
	Lot 3		Lot 3
MM Specimen 4	Lot 1	MM Specimen 9	Lot 2
	Lot 2		Lot 3
MM Specimen 5	Lot 1		
	Lot 3		

The percentage of cells with an abnormal FISH signal pattern(s) for each probe are listed in Table 8 are shown in bold. Three (3) BMA gave normal FISH signal patterns for all five probes tested. One BMA was abnormal for the t(11;14) translocation and 4 BMA had abnormal signal patterns for the 13q deletion. In all nine BMA tested, the percentage of abnormal cells for each probe were very similar between the plasma cells enriched with the two EasySep Human Bone Marrow CD138 Positive Selection Kit lots. The same normal/abnormal disposition for a given probe was concordant between the pairs of EasySep Human Bone Marrow CD138 Positive Selection Kit lots. Cells which are bold in Table 8 were dispositioned as abnormal with abnormal FISH signal patterns. Cells which are not bold were dispositioned as normal with abnormal FISH signal patterns that did not exceed the cutoffs for percentage of abnormal nuclei. In all cases, normal/abnormal disposition agreed, indicating that the EasySep Human Bone Marrow CD138 Positive Selection Kit is able to produce reproducible FISH results when used on the intended use specimen.

Table 8. FISH Enumeration Results Using Five Probes on MM BMAs Following Enrichment Using the EasySep Human Bone Marrow CD138 Positive Selection Kit

Sample No.	Kit Lot	t(11;14)	Trisomy			Tetrasomy			Monosomy			13q
		% Abnormal	Chr.5	Chr.9	Chr.15	Chr.5	Chr.9	Chr.15	Chr.5	Chr.9	Chr.15	Deletion
1	1	0	**25.5**	**14**	4.5	6.5	1	1	0.5	2	3.5	4
1	2	0	**27**	**18**	6.5	1.5	2	0.5	1.5	1	1	5
2	1	0	0	**88**	0	0	1.5	0	0	0	10	**96**
2	3	0	1	**87.5**	0	0	0.5	0.5	1.5	0	7.5	**95.5**
3	2	0	**74.5**	**55.5**	**49**	0.5	0	0	0	0	0	**84.5**
3	3	0	**72**	**59**	**46.5**	0	2.5	1	0	0	0	**80**
4	1	0	2	1	1.5	0	0	0	2.5	2.5	1	2
4	2	0	3	3	1	0	0	0	1.5	4	5.5	2.5
5	1	0	9.5	5.5	6.5	0.5	1	1	6	2.5	5	2.5
5	3	0	3	4.5	2	0.5	0.5	0.5	2	2	4	2.5
6	2	0	2	0	**53**	0	0	0	0.5	0	1	**96.5**
6	3	0	0.5	1.5	**38.5**	0	1	0	0.5	2	3	**95.5**
7	1	**89**	0	2	2	0	0	0	0	5.5	4	**90.5**
7	2	**90**	2.5	7	2	0	0	0.5	2	1	4	**91**
8	1	0	0.5	1.5	1	0	1	0	2	1.5	4	1
8	3	0	1.5	0.5	1	2.5	0	0	1.5	1	3	0.5
9	2	0	**63**	**53**	**35**	5	1.5	4.5	1.5	1	0	2
9	3	0	**60**	**48.5**	**38.5**	1	0	0	1	1.5	3.5	1

2. Repeatability

In this precision study, one operator enriched a low or medium initial CD138+ frequency panel member using one EasySep Human Bone Marrow CD138 Positive Selection Kit lot in quadruplicate over three runs using contrived samples. On three non-consecutive days, a bone marrow specimen from a healthy donor was spiked with the CD138+ cell line SK-MM-2 at a low (< 3%) and a medium (3 - 15%) frequency to generate two separate contrived sample panel members. Two operators performed EasySep enrichments on each of the study days, with each operator enriching from one of the two panel members. Each operator performed three runs of enrichments in quadruplicate on one panel member using one GMP kit lot.

Table 9 shows the unenriched and enriched CD138+ cell purity from all panel members and enriched CD138+ cell purity from all panel members across all three runs. Low initial CD138+ frequency panel members had mean CD138+ cell purities ranging from 1.32 - 1.51% before enrichment and increased to CD138+ cell purities of 80.99% - 87.93% after EasySep enrichment. Medium initial CD138+ frequency panel members had CD138+ cell purities ranging from 9.84% - 10.84% before enrichment and increased to mean CD138+ cell purities of 91.10% - 96.65% after EasySep enrichment.

Table 9. Unenriched and Enriched CD138+ Cell Purity for Low and Medium Initial CD138+ Frequency Panel Members as Determined by Flow Cytometry Analysis

Initial CD138+ Frequency Category	Panel Member	Unenriched Purity* (% CD138+)	Enriched Purity (%CD138+)					
			Mean	Min	Max	SD	CV	N
Low	B1	1.51	84.91	83.05	87.48	1.51	1.77	12
	B4	1.32	86.77	85.65	87.93	0.73	0.84	12
	B5	1.37	82.41	80.99	83.77	0.93	1.13	12
Medium	B2	10.84	93.82	92.75	95.62	0.90	0.96	12
	B3	9.84	95.70	94.81	96.65	0.62	0.65	12
	B6	10.17	93.32	91.10	94.21	0.77	0.83	12

Table 10 shows the CD138+ cell recovery for low and medium initial CD138+ frequency panel members and the corresponding statistical analyses. Low initial CD138+ frequency panel members had CD138+ cell recoveries ranging from 34.98% - 76.69% (Mean = 53.42%). Medium initial CD138+ frequency panel members had CD138+ cell recoveries ranging from 36.89% - 65.16% (Mean = 50.62%). The overall CD138+ cell recovery within-laboratory precision estimates (%CV across three runs with four replicates, n = 12 total) ranged from 5.52% - 27.4% and 10.53% - 18.32% for low and medium initial CD138+ frequency panel members respectively.

Table 10. CD138+ Cell Recovery for Low and Medium Initial CD138 Frequency Panel Members

Initial CD138 Frequency Category	Panel Member	Unenriched Purity* (% CD138+)	CD138+ Cell Recovery (%)					
			Mean	Min	Max	SD	CV	N
Low	B1	1.51	55.30	34.98	76.69	15.15	27.4	12
	B4	1.32	48.88	36.63	64.37	7.92	16.2	12
	B5	1.37	56.09	50.56	63.00	3.09	5.52	12
Medium	B2	10.84	50.78	37.58	58.57	6.64	13.07	12
	B3	9.84	49.04	36.89	57.99	5.16	10.53	12
	B6	10.17	52.04	37.88	65.16	9.53	18.32	12

* Mean unenriched purity measured across three runs

Table 11 shows the FISH analysis results for the selected enriched samples from each panel member and run. For low initial CD138+ frequency panel members, the number of abnormal nuclei per slide ranged from 85.5% - 94.0% and replicates from all panel members and runs resulted in abnormal FISH signal patterns. For medium initial CD138+ frequency members, the number of abnormal nuclei per slide ranged from 93% -99.5%, and replicates from all panel members and runs resulted in abnormal FISH signal patterns. Therefore, the acceptance criteria for within laboratory precision by FISH analysis were met.

Table 11. FISH Analysis Results for Enriched Samples by Run for Low and Medium Initial CD138 Frequency Panel Members

Initial CD138 Frequency Category	Panel Member	Run	Replicate (a/b/c/d)	CD 138 Purity (% CD138+)		FISH Results	
				Unenriched	Enriched	# Abnormal Nuclei	Normal/ Abnormal
Low	B1	1	b	1.51	87.48	92.5	Abnormal
		2	d		83.42	94	Abnormal
		3	a		84.41	93	Abnormal
	B4	1	d	1.32	85.65	90	Abnormal
		2	c		87.93	89.5	Abnormal
		3	a		87.44	94	Abnormal
	B5	1	b	1.37	80.99	85.5	Abnormal
		2	c		83.77	86	Abnormal
		3	d		82.12	89.5	Abnormal
Medium	B2	1	a	10.84	95.62	99.5	Abnormal
		2	b		93.57	96.5	Abnormal
		3	d		92.75	97	Abnormal
	B3	1	b	9.84	95.43	94.5	Abnormal
		2	d		94.81	95.5	Abnormal
		3	c		96.14	94	Abnormal
	B6	1	a	10.17	91.1	93	Abnormal
		2	a		93.69	93.5	Abnormal
		3	b		94.21	97.5	Abnormal

The CD138 EasySep Precision study showed that the EasySep Human Bone Marrow CD138 Positive Selection Kit produced consistent enrichment within a run and across three runs when starting with a specimen of a given volume and initial CD138+ cell frequency. For low initial CD138+ frequency panel members (n=3) flow cytometry analysis, the repeatability precision estimate (n=4 per run) for enriched CD138+ purity CV ranged from 0.81% to 1.56% and the within-laboratory precision estimate (n=12 across 3 runs) for enriched CD138+ purity CV ranged from 0.84% to 1.77%. For medium initial CD138+ frequency panel members (n=3) flow cytometry analysis, the repeatability precision estimate (n= 4 per run) for enriched CD138+ purity CV ranged from 0.17% to 1.41% and the within-laboratory precision estimate (n=12 across 3 runs) for enriched CD138+ purity CV ranged from 0.65% to 0.96%. All enriched samples sent for FISH analysis (n=3 per panel member, one replicate per run) gave abnormal FISH patterns, demonstrating within-laboratory precision in downstream FISH assays. Overall, the precision of the EasySep Human Bone Marrow CD138 Positive Selection Kit met the acceptance criteria set for the precision study.

3. Analytical Specificity/Interference:

Three (3) fresh clinical multiple myeloma bone marrow specimens were tested to determine if excess heparin affected device performance. One specimen was processed at ~20 hours post collection, one specimen was processed at ~24 hours post collection, and the third specimen was processed ~22 hours post collection. Each specimen was split into two fractions. One fraction was treated with phosphate buffered saline (PBS, the control), while the other was spiked with 3x excess of sodium heparin. Single replicates of each fraction were then washed and enriched using the EasySep Human Bone Marrow CD138 Positive Selection Kit. CD138 purity was determined by flow cytometry and then

compared to determine whether excess heparin caused interference with CD138 enrichment and purity. The enriched CD138+ purity from the test samples with excess sodium heparin should be ≥ 90% of the enriched CD138+ purity from the control samples without excess heparin.

Table 12 shows CD138 purity before plasma cell enrichment and after enrichment using the EasySep Human Bone Marrow CD138 Positive Selection Kit for each specimen. For specimen #1, the PBS control sample was enriched to 64.7% and the excess sodium heparin-containing sample was enriched to 58.7%, which is 91.0% of the control. For specimen #2, the PBS control sample was enriched to 32.4% and the excess sodium heparin containing sample was enriched to 33.4%, which is 103% of the PBS control. For specimen #3, the PBS control sample was enriched to 81.7% and the excess sodium heparin-containing sample was enriched to 80.1%, which is 98.0% of the control. All three specimens passed the set acceptance criteria for the study, which was that enriched CD138+ purity from the test samples with excess sodium heparin should be ≥ 90% of the enriched CD138+ purity from the control samples without excess heparin.

Table 12. Effect of Excess Heparin on CD138 Purity

Specimen #	Initial CD138 Frequency (%)	After Enrichment		CD138 Purity: Heparin/ PBS Control	Meets Acceptance Criteria? (Pass/Fail)
		PBS Control (%)	+ Heparin (%)		
1	5.0	64.7	58.7	91.0	PASS
2	0.4	32.4	33.4	103.0	PASS
3	31.3	81.7	80.1	98.0	PASS

A second study evaluated a total of 3 clinical specimens prepared by adding MM bone marrow mononuclear cells into healthy donor bone marrow at <0.2% initial CD138 frequency. Three (3) replicates were tested per specimen per condition (i.e., with/without excess sodium heparin). The enriched purities of heparin spiked specimens were not statistically different from that of the control specimens (one tailed t-test, p=0.2740).

Table 13 shows CD138 purity before plasma cell enrichment and after enrichment using the EasySep Human Bone Marrow CD138 Positive Selection Kit for each specimen. The mean enriched purity for Clinical Specimen 1 was 84.45% for the Control Specimen and 83.97% for the Heparin-spiked Specimen, which was 99% of Control. The mean enriched purity for Clinical Specimen 2 was 96.37% for the Control Specimen and 96.25% for the Heparin-spiked Specimen, which was 100% of Control. The mean enriched purity for Clinical Specimen 3 was 88.90% for the Control Specimen and 89.08% for the Heparin-spiked Specimen, which was 100% of Control. The enriched purities of heparin spiked specimens were not statistically different from the enriched purities of control specimens (one tailed t-test, p=0.2740, N=3). All three clinical specimens passed the acceptance criteria that enriched CD138+ purity from the test samples with excess sodium heparin should be ≥ 90% of the enriched CD138+ purity from the control samples without excess heparin.

Table 13. Summary of CD138 Purity from Low Frequency Clinical Specimens

Clinical Specimen	Target Initial % CD138	Replicate	Enriched Purity (% CD138+)		Mean Enriched Purity (% CD138+)		Heparin/ Control (%)	Acceptance Criteria (Pass/Fail)
			Control	Heparin	Control	Heparin		
1	0.14	1	83.52	81.69	84.45	83.97	99	Pass
		2	85.26	83.23				
		3	84.57	86.99				
2	0.14	1	96.45	96.26	96.37	96.25	100	Pass
		2	96.74	96.21				
		3	95.92	96.27				
3	0.19	1	89	90.05	88.90	89.08	100	Pass
		2	88.79	89.24				
		3	NA	87.96				

Results show the enriched purities from the excess anticoagulant containing samples were not statistically different from those of the control samples. Therefore, the study results demonstrate that there is no interference identified from the excess anticoagulant used when enriching samples with the EasySep Human Bone Marrow CD138 Positive Selection Kit.

4. Stability

Specimen Stability

The CD138 Expression Stability Study aimed to process ~six MM patient BMA at baseline (<24 hours) and at either 44 - 54 hours or ~73 hours post-draw. One kit lot of the EasySep Human Bone Marrow CD138 Positive Selection kit was used for each time point. At each time point, flow cytometry was performed for both unenriched and enriched portions of each sample, and FISH was only performed on the enriched portion of each sample. For FISH analysis, all enriched samples should have conclusive FISH results. Each of the five FISH probes should result in the same FISH disposition (i.e., normal or abnormal).

CD138 purity before and after enrichment and FISH results using five common FISH probes for each time point are shown in Table 14. Specimen #1 had an initial CD138 frequency of 10.6%, which dropped to 3.1% at 73 hours post-collection. Following enrichment, plasma cells were enriched to 89.9% at baseline, and decreased to 62.4% at 73 hours. FISH results for the three probes analyzed did not change across the time points. Specimen #2 had a very low initial CD138 frequency of 0.5%, which decreased below the limit of quantitation (LLoQ) for the flow assay at 44 - 54 hours and further decreased to below the limit of detection (LLoD) for the flow assay at 73 hours post-collection. As such, data are reported as <LLoQ and <LLoD. FISH results were consistent with baseline and 44 - 54 hours, but were more variable at ~73 hours, as there were insufficient cells to obtain conclusive FISH results for two of the probes. The initial CD138 frequency could not be determined at this time point and could therefore be below the LoD of 0.05% that has been determined for the EasySep Human Bone Marrow CD138 Positive Selection Kit. Specimen #3 also had a very low initial CD138 frequency of 0.4%. At ~73 hours post-collection, purity was again below the LLoD for the flow assay and could not be determined. FISH disposition for the three probes analyzed was consistent across the time points.

Table 14. Stability of CD138 Expressing Multiple Myeloma Bone Marrow Aspirates

Bone Marrow Specimen	FISH Probe	Baseline (<24 h)	44 - 54 h	~73 h
1	Initial CD138 Frequency	10.6%	9.1%	3.1%
	Enriched CD138 Purity	89.9	85.1	62.4
	Chr. 13 (D13S319)	Abnormal	Abnormal	Abnormal
	CCND1/IGH XT	Normal	Normal	Normal
	TP53	Normal	Normal	Normal
2	Initial CD138 Frequency	0.5%	<LLoQ** (not quantifiable)	<LLoD** (not detected)
	Enriched CD138 Purity	36.7	30.8	28.9
	Chr. 13 (D13S319)	Abnormal	Abnormal	QNS
	CCND1/IGH XT	Normal	Normal	Normal
	TP53	Normal	Normal	QNS
3	Initial CD138 Frequency	0.4%	Not tested	<LLoD** (not detected)
	Enriched CD138 Purity	32.4	Not tested	23.2[&]
	Chr. 13 (D13S319)	Normal	-	Normal
	CCND1/IGH XT	Normal	-	Normal
	TP53	Normal	-	Normal

*QNS - Quantity of cells not sufficient for analysis
**CD138 purity values are below the putative LLoQ of 0.4% or LLoD of 0.14% for the flow cytometry assay performed and could not be determined.
[&]Note: This sample was processed at 69 hours post-collection instead of 73 hours.

Overall, FISH disposition was consistent between baseline and ~73 hours for all three specimens when initial CD138 frequencies were above the putative LLoD for the flow assay (i.e., 0.14%). Although CD138 specimens could be enriched at ~73 hours, FISH signals were not reliable when specimens with low initial CD138 frequencies were too low to be determined to be determined by flow cytometry.

Kit Stability

Shelf-Life and In-Use

This study was conducted to establish that the EasySep Human Bone Marrow CD138 Positive Selection Kit is able to maintain its performance characteristics over its defined shelf-life when stored at 5°C ± 3°C using three kit lots. The test samples were stored unopened at 5°C ± 3°C. Contrived samples were tested at or to be test at different time points (0, 3, 7, 10, 13, 19 and 25 months). Three kit lots were tested for shelf-life and 1 lot was tested for in-use stability. The in-use testing involved the mixing and removal of 25 µL from the cocktail and particle vials for 20 times over a 7-month duration before the samples were tested. Kits had to demonstrate ability to enrich CD138+ cells to a purity of ≥95% and ≥70% recovery.

Stability testing of three lots of EasySep Human Bone Marrow CD138 Positive Selection Kit has met all acceptance criteria at the long-term and in-use storage condition of 5°C ± 3°C for up to 7 months and at the accelerated storage condition of 25°C ± 2°C/ 60% ± 5% RH for up to 7 days. The data therefore support a 6-month shelf life for EasySep Human Bone Marrow CD138 Positive Selection Kit stored at 5°C ± 3°C. The data also

supports the stability of the products for up to 7 days at 25°C ± 2°C/ 60% ± 5% relative humidity.

Shipping

This study summarizes data collected for up to 7 months at the long-term storage condition of 5°C ± 3°C from the shipping stability study conducted on one batch of EasySep Human Bone Marrow CD138 Positive Selection Kit. Shipping stability kits packaged in their final packaging configurations were subjected to summer and winter temperature profiles for 48 hours and tested for enrichment performance and FISH and monitored with data loggers. For the 48-hour Summer Profile Testing, the test article was exposed to temperature test cycles with a recorded maximum temperature of 30.5 °C and minimum of 23.8 °C. For the 48-hour winter profile testing, the maximum recorded temperature to which the test article was exposed to is 15.1°C while the minimum recorded temperature is 3.6 °C. Kits had to demonstrate that CD138+ cell purity had to be >95% with at least 70% of cells recovered. All acceptance criteria were met at the long-term storage condition of 5°C ± 3°C for up to 7 months.

The stability data demonstrated that transport of the EasySep Human Bone Marrow CD138 Positive Selection Kit using the qualified 48-hour shipping configuration does not affect product performance characteristics. The data support a 6-month shelf-life following transport stress. The shipping stability study will continue for up to 25 months.

5. Detection Limit:

a) Limit of Detection with Contrived Samples

The study aimed to determine the lowest initial CD138+ frequency that gives consistent abnormal FISH results in a contrived specimen after CD138+ cell enrichment using the EasySep Human Bone Marrow CD138 Positive Selection Kit. On six non-consecutive days, a whole blood (WB) or BM sample from a healthy donor was spiked with the CD138+ cell line SK-MM-2 at 20% (acceptable range 26% -14% by flow cytometry) CD138+ initial frequency and then serially diluted by a factor of two with WB or BM to achieve ~20%, 10%, 5%, 2.5%, 1.25%, 0.63%, 0.31%, 0.16%, 0.08%, and 0% (i.e., WB or BM not spiked with SK-MM-2 cells). Each sample was enriched in duplicate using the EasySep Human Bone Marrow CD138 Positive Selection Kit and all unenriched and enriched specimens were assessed by flow cytometry.

Table 15 shows the results from the EasySep CD138 Limit of Detection Study including the unenriched and enriched CD138+ cell purity measured by flow cytometry and the t(11;14) disposition analyzed by FISH. All contrived specimens spiked with SK-MM-2 cells, targeting as low as 0.08% initial CD138+ frequency, resulted in abnormal t(11;14) disposition in FISH analysis.

The enriched CD138+ purity measured by flow cytometry positively correlated with the percentage of abnormal nuclei observed in downstream FISH analysis. The average enriched CD138+ purity that resulted in abnormal t(11;14) disposition in FISH was 33% CD138+, enriching from specimens with an average unenriched purity of 0.07% CD138+. For 0.00% initial target CD138+ frequency specimens, four out of the six donors resulted in normal t(11;14) disposition in FISH analysis, however one BM and one WB donor only had one replicate deemed normal and the other replicate inconclusive

due to insufficient number of cells recovered. Since the lowest targeted initial CD138+ frequency specimen achieved abnormal t(11;14) disposition by FISH, the limit of detection (i.e., the lowest initial CD138+ frequency in a contrived specimen that gave a consistent abnormal t(11;14) disposition in FISH after CD138+ enrichment) was determined to be in the range of 0.05 - 0.15% CD138+ measured by flow cytometry. The lower end of the range was below the limit of detection of the flow cytometer. Therefore, the limit of detection was set at the limit of detection of the flow cytometer, which was 0.05% CD138+.

Table 15. EasySep CD138 Limit of Detection Flow Cytometry and FISH Analysis Result Summary

Target CD138+ Initial Frequency	Flow Cytometry Analysis								FISH Analysis				
	Unenriched CD138+ Purity (%)				Enriched CD138+ Purity (%)				%Abnormal Nuclei and t(11;14) Disposition				
	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max	Normal/ Abnormal	N
0.00%	NA	ND	<LoQ	ND: 5 <LoQ: 1	13.33	<LoQ	32.48	12	0.0	0.0	0.0	Normal Inconclusive	N=10 N=2
0.08%	0.09*	0.05*	0.15*	6	33.03	6.31	68.07	12	51.7	30.5	73.0	Abnormal	12
0.16%	0.18*	0.10*	0.30*	6	42.57	11.52	76.2	12	68.0	40.0	82.0	Abnormal	12
0.31%	0.32*	0.21*	0.40*	6	55.42	20.73	83.82	12	83.4	73.5	90.5	Abnormal	12
0.63%	0.69	0.41	0.94	6	67.2	30.97	90.12	12	87.6	80.0	92.5	Abnormal	12
1.25%	1.38	0.87	1.86	6	76.55	51.55	90.43	12	93.1	85.5	97.0	Abnormal	12
2.50%	2.17	1.41	2.84	6	87.07	76.24	93.86	12	95.8	89.5	99.0	Abnormal	12
5%	5.28	3.37	7.25	6	91.71	88.26	95.01	12	97.0	93.5	100.0	Abnormal	12
10%	10.71	8.16	13.21	6	94.39	91.2	96.91	12	98.0	96.5	99.5	Abnormal	12
20%	20.01	14.73	25.06	6	95.01	92.93	98.2	12	98.1	96.5	100.0	Abnormal	12

Table 16 shows the unenriched and enriched purities for BM and WB contrived specimens for each target initial frequency. The healthy donor BM only specimens (0% initial CD138+ frequency) had enriched CD138+ purities in the range of 16.7% - 32.5%.

Table 16. Unenriched and Enriched Purities for BM and WB Contrived Specimens for Each Target Initial Frequency

Target Initial CD138+ Frequency	Sample Type							
	BM				WB			
	Unenriched Purity (% CD138+)	Enriched Purity (% CD138+)			Unenriched Purity (% CD138+)	Enriched Purity (% CD138+)		
	Mean	Mean	Min	Max	Mean	Mean	Min	Max
0	0.04	26.21	16.66	32.48	0.00	0.46	0.10	0.98
0.08	0.07	54.67	39.73	68.07	0.07	11.40	6.31	16.10
0.16	0.18	65.4	54.28	76.2	0.12	19.75	11.52	33.01
0.31	0.31	75.61	69.86	83.82	0.21	35.23	20.73	55.57
0.63	0.71	82.35	74.86	90.12	0.67	52.05	30.97	72.51
1.25	1.29	85.35	78.84	90.43	1.47	67.74	51.55	81.51
2.5	2.33	89.78	83.92	93.86	2.01	84.37	76.24	91.95
5	5.82	91.87	89.21	95.01	4.74	91.56	88.26	93.93
10	10.41	93.75	91.20	95.94	11.01	95.03	92.83	96.91
20	19.84	94.34	92.93	96.87	20.18	95.68	94.06	98.2

b) Limit of Detection with Clinical Multiple Myeloma Bone Marrow Specimens

Five (5) low CD138 frequency specimens with pre-enrichment frequencies of 0.2%, 0.5%, 0.7%, 1.5%, and 1.7% CD138+ (Table 17). All five specimens were successfully enriched to purity ≥ 18.5% CD138+ with fold enrichment ≥ 29.6-fold. All five specimens had an increase in the percentage of abnormal nuclei in FISH analysis for at least one FISH probe. Three of the five specimens only had percentages of abnormal nuclei above FISH cutoff after plasma cell enrichments (highlighted in bold). The data confirm that clinical MM BM specimens with initial CD138+ frequency close to the limit of detection set in this study were able to have plasma cells successfully enriched and gave interpretable FISH results.

Table 17. CD138 Purity and Percentage of Abnormal Nuclei Detected by FISH Probes Pre- and Post-EasySep CD138 Enrichment for Low CD138 Initial Frequency Multiple Myeloma Bone Marrow Aspirates

MM Patient Sample	CD138 Freq*	Pre- Enr CD138 Purity	Enr CD138 Purity	Fold Enr&	% Abnormal Nuclei											
					Probe (D5S23) Cutoff = 1%		Probe (D15Z4) Cutoff = 1%		Probe (MYC) Cutoff = 3%		Probe (D13S319, 13qter) Cutoff = 14%		Probe (IGH/CCND1 XT) Cutoff = 3-15%		Probe (TP53, D17Z1) Cutoff = 9%	
					Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr	Pre- Enr	Enr	Pre-Enr	Enr
BM 118	low	0.7	64.5	91.1	0	0	0	0	0	0	0	0	4	85.5	0	0
BM 121	low	0.2	18.5	91.5	1.5	48	0	0	0	0	**0**	**50**	0	0	0	0
BM 122	low	1.7	52.0	29.6	0	0	0	0	0	0	**0**	**80**	0	0	0	0
5	low	1.5	80.6	52.7	0	0	**0**	**4**	0	0	**0**	**49**	**0**	**77**	0	0
8	low	0.5	32.8	64.6	5	53	0	0	0	0	0	0	0	0	0	0

Enr = enrichment

*low = <3% CD138 start frequency

& Fold enrichment is calculated using the following formula: [Enriched %CD138 Purity / Pre-enriched %CD138 Purity] - 1

The limit of detection study showed that the EasySep Human Bone Marrow CD138 Positive Selection Kit was able to consistently enrich from contrived specimens with initial CD138+ frequency of 0.02% - 25.06% and achieved consistent abnormal FISH patterns even in the lowest initial target CD138+ frequency specimens spiked with the SK-MM-2 cell line. Therefore, the limit of detection for the EasySep Human Bone Marrow CD138 Positive Selection Kit was set to 0.05% of initial CD138+ frequency, which is the limit of detection of the flow cytometer used.

c) Verification of Limit of Detection with Clinical Multiple Myeloma Bone Marrow Specimens

The goal of this study was to verify the LoD at 0.05% initial CD138 frequency, determined in a previous LoD study on contrived specimens in one clinical specimen using two kit lots, having 3 replicates per kit lot. CD138+ cell enrichment was performed on a clinical specimen produced by spiking a multiple myeloma patient BM aspirate into a healthy donor BM aspirate at 0.05% initial CD138 frequency. The clinical specimen was washed and split into 6 fractions for EasySep CD138 cell enrichments, using two kit lots with three replicates per kit lot. Enriched samples were split into two fractions: one fraction was used for plasma cell purity assessment by flow cytometry and the second fraction was used for FISH.

Table 18 shows plasma cell purity before and after enrichment using two kit lots of the EasySep Human Bone Marrow CD138 Positive Selection Kit with three replicates per kit lot. The specimen was enriched from 0.05% to a range of 4.1% to 7.4%. Overall enriched purity CV was 19.7%, passing the acceptance criteria of overall CV ≤ 35%. All enriched samples had normal FISH signal patterns for Chromosome 5 and 15 polysomy (D5S23, D15Z4), MYC breakapart, and the CCND1/IGH XT probes; and were abnormal for Chromosome 13q deletion (D13S319) and TP53. The enriched samples' FISH disposition was the same as the undiluted clinical specimen's FISH disposition, with 100% of the replicates being abnormal for Chromosome 13q deletion (D13S319) and TP53.

Table 18. Summary of Limit of Detection Results for Clinical Specimens

Sample	Unenriched Plasma Cell Purity (% per 10^5 events)	Enriched Plasma Cell Purity by Flow Cytometry				Genomic Abnormality by FISH					
		% Plasma Cell per 10^5 events	Mean	SD	%CV	D5S23	D15Z4	MYC	D13S319, 13qter	TP53, D17Z1	IGH/CCND1 (LDT)
Undiluted MM BMA	12.9	-	-	-	-	Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 1 - A	0.05	4.1	6.2	1.2	19.7	Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 1 - B		7.4				Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 1 - C		7.0				Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 2 - A		5.8				Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 2 - B		7.0				Normal	Normal	Normal	Abnormal	Abnormal	Normal
Lot 2 - C		5.8				Normal	Normal	Normal	Abnormal	Abnormal	Normal

B Clinical Studies:

1. Clinical Study

This study summarizes CD138 purity data and FISH data generated on bone marrow aspirates (BMA) from 33 multiple myeloma patients at various stages of disease collected in two studies. In both studies, one portion of the sample was retained as the pre-enriched sample, and the other portion was split again and enriched with the EasySep Human Bone Marrow CD138 Positive Selection Kit. CD138 purity was assessed by flow cytometry on both the pre-enriched and enriched samples. Each sample (pre-enriched and enriched) was then hybridized with five common FISH probe kits that included the CCND1/IGH XT Probe Kit to detect the t(11;14) translocation, as well as probes to detect chromosome 5 (D5S23) and chromosome 15 polysomy (D15Z4) (single probe kit), MYC breakapart, 13q deletion (D13S319), and TP53 deletion (D17Z1).

Specimen slides were enumerated by a minimum of two readers; 50 nuclei per reader were analyzed, for a total of 100 nuclei. If counts for a reader were below or equal to the cutoff corresponding to each probe, then the result from the reader was determined to be normal and the percentage of abnormal nuclei would be indicated as '0' (Table 19).

Thirty-three BMAs from patients at various stages of disease were enriched using the EasySep Human Bone Marrow CD138 Positive Selection Kit. CD138 purity was determined by flow cytometry and the percentage of abnormal nuclei detected for five common FISH probes for all pre-enriched and enriched specimens. CD138 purity data

pre and post EasySep enrichment was collected by flow cytometry for 30 of the 33 patients. CD138 purities prior to enrichment ranged from 0.2% to 82.7% (average 12.9%); five specimens had CD138+ plasma cell purities of <3% prior to enrichment, 16 had purities between 3% and 15%, and nine had purities >15%. Purities after EasySep enrichment ranged from 18.5% to 98.6% (average 79.6%).

Table 19. CD138 Purity and Percentage of Abnormal Nuclei Detected or Five FISH Probes Pre- and Post-EasySep CD138 Enrichment for 33 Multiple Myeloma Bone Marrow Aspirates

MM Patient Sample	CD138 Frequency*	Pre-Enr CD138 Purity	Enriched CD138 Purity	Fold Enrich-ment&	% Abnormal Nuclei											
					Probe (D5S23)		Probe (D15Z4)		Probe (MYC)		Probe (D13S319, 13qter)		Probe (IGH/ CCND1 XT)		Probe (TP53, D17Z1)	
					Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr	Pre-Enr	Enr
1	medium	5.0	57.3	10.5	0	0	0	0	0	0	0	51	13	53	0	0
2	high	15.5	91.4	4.9	35.5	87	21.5	63	0	0	0	0	0	0	0	0
3	high	15.6	98.2	5.3	0	0	0	0	0	17	19	88	0	0	0	0
4	high	82.7	77.7	-0.1	38	45	34.5	30	69	91	50.5	74	0	0	54	70
5	medium	4.3	57.0	12.3	25.5	74	28.5	67	0	0	0	0	0	8.5	0	0
6	medium	6.5	98.6	14.2	0	0	19	44	25.5	46	14.5	73	32.5	92	26.5	77
7	medium	10.0	94.4	8.4	20	69	17.5	71	0	0	0	0	0	0	0	0
8	high	36.2	93.7	1.6	45	88	0	0	39	53	0	0	54	94	0	0
9	medium	13.8	92.0	5.7	0	0	0	0	29.5	89	24.5	89	0	0	0	0
10	high	31.4	85.7	1.7	0	0	9	75	0	0	0	90	18	95.5	0	0
11	medium	7.9	85.6	9.8	0	0	0	0	0	0	0	95	9.5	91.5	0	0
12	high	16.2	91.0	4.6	29.5	85	16	51	0	0	41	93	21.5	86.5	0	0
13	medium	3.7	88.9	23.0	0	0	0	0	0	0	0	0	0	0	0	0
14	medium	12.4	75.3	5.1	14.5	47	0	0	23.5	88	24	97	0	0	9.5	43
15	medium	7.7	81.7	9.6	0	0	17	53	0	6	20.5	93	0	8.5	0	0
16	high	35.0	95	1.7	0	0	0	0	0	0	40	88	0	0	46.5	81
17	medium	4.6	82.9	17.0	0	0	0	0	0	0	18	77	8	82	0	22
18	low	0.7	64.5	91.1	0	0	0	0	0	0	0	0	4	85.5	0	0
19	medium	4.9	88.3	17.0	10.5	75	7	27	0	0	0	0	0	0	0	0
20	high	27.7	96.8	2.5	77	91	68	81	0	0	0	0	0	0	0	0
21	low	0.2	18.5	91.5	1.5	48	0	0	0	0	0	50	0	0	0	0
22	low	1.7	52.0	29.6	0	0	0	0	0	0	0	80	0	0	0	0
23	high	17.2	NC	n/a	15	95	16	93	0	0	0	0	29	92	0	0
24	medium	5.7	NC	n/a	0	0	0	0	0	0	0	90	0	0	0	0
25	low	2.3	NC	n/a	0	0	0	0	0	0	0	0	7	64	0	0
26	medium	10.6	96.3	8.1	8.5	68	9	73	7.5	37	0	0	0	0	0	0
27	low	1.5	80.6	52.7	0	0	0	4	0	0	0	49	0	77	0	0
28	high	20	92.1	3.6	0	0	4	61	0	0	40	91	0	0	0	0
29	medium	9.7	90.6	8.3	21.5	75	13.5	55	0	8	18.5	84	0	0	0	0
30	low	0.5	32.8	64.6	5	53	0	0	0	0	0	0	0	0	0	0
31	medium	5	84.6	15.9	0	0	0	0	0	0	0	0	4	83	0	0
32	medium	5.8	87.5	14.1	0	0	0	0	0	0	0	80	0	0	0	0
33	medium	4.3	56.2	12.1	28	91	28	87	18	72	0	96	0	0	0	0

Enr = enriched
*low = <3% CD138 start frequency; medium = 3-15% CD138 start frequency; high = >15% CD138 start frequency
& Fold enrichment is calculated using the following formula: [Enriched %CD138 Purity / Pre-enriched %CD138 Purity] - 1 NC - not collected

CD138 purity increased from 12.9% ± 15.9% in the pre-enriched sample to 79.6% ± 19.9% following enrichment. Fold enrichment was > 1 for all specimens with CD138 frequencies <40% prior to enrichment. In 14 patients, abnormal nuclei were only detected after the sample had been enriched using the CD138 kit (bold). For all patients, the percentage of abnormal nuclei detected was higher in the enriched sample compared to the unenriched sample indicating an increase in the sensitivity of the FISH assay. This data demonstrates that the EasySep Human Bone Marrow CD138 Positive Selection kit can be used to enrich CD138+ plasma cells from multiple myeloma patients for use in detection of multiple FISH probes.

VII Proposed Labeling:

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

VIII Identified Risks and Mitigations:

Risks to Health	Mitigation Measures
Failure to perform as expected due to errors in enrichment, contributing to false positive or false negative results, or failure to produce results in downstream assays	Use of certain specimen collection devices. 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 enrichment results by the lab.	Certain labeling information, including limitations, device descriptions, methodology and protocols, and performance information. Certain design verification and validation, including certain studies and risk mitigation analysis.

IX Benefit/Risk Assessment:

A Summary of the Assessment of Benefit:

The EasySep Human Bone Marrow CD138 Positive Selection Kit has probable benefit given the totality of data provided, which indicate that use of this device provides enriched and purified CD138+ plasma cells (both benign and malignant), for downstream applications like molecular assays and flow cytometry. In addition, in the clinical studies, the sponsor was successfully able to enrich a range of bone marrow CD138+ cells (low, intermediate, and high), enriching the CD138+ cells with their device, and showed that they were able to successfully perform FISH with these viable, enriched cells. A key benefit of this device is that it increases the concentration of the cells of interest in samples with low concentrations of specific hematopoietic cells, such as those in patients undergoing treatment or relapsed refractory patients, which will help decrease the false negative rate for any test. Patients and

physicians will have a better idea of the molecular genetics of their disorder, because this selection kit allows for enrichment of CD138+ plasma cells, such that cytogenetic and molecular analysis can be performed with enriched cells. This may lead to better clinical management for patients with multiple myeloma or other hematopoietic malignancies.

B Summary of the Assessment of Risk:

The probable risks of this device include the malfunction of CD138+ plasma cell purification, which may impact downstream assays, are: 1) failure to provide a result due to errors in enrichment, contributing to false positive or false negative results or failure to produce results in assays and 2) incorrect interpretation of enrichment results by the lab. Poor enrichment of plasma cells may lead to false negative results, or no results reported. There is also a degree of probable risk regarding false positives; however, the risk of false positivity is thought to be low, due to technical reasons.

In the validation of this device, not all downstream assays were tested. The user is responsible for the adequate validation of downstream assay. The device has limitations where very low levels of malignant plasma cells may not be able to be sufficiently enriched for downstream applications.

The risks outlined above, are mitigated by use of specified specimen collection devices. substantive design verification and validation studies (including supportive analytical and clinical studies), labeling information, including limitations, device descriptions, methodology and protocols, and performance information. In addition, a risk mitigation analysis was performed, indicating that the major sources of risk were adequately mitigated.

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 of cell viability after enrichment and studies that showed that the enriched cells yielded interpretable results from downstream molecular analysis. The limitations of the enrichment and potential variability in enrichment were also shown. 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 and serve to mitigate risks of this device. Therefore, while general controls are not sufficient to address the risks of this device, when taking into account the special controls, the probable benefits of this device are deemed to outweigh the probable risks of this device.

X 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(s): QYO
Device Type: Hematopoietic cell enrichment kit
Class: Class II
Regulation: 21 CFR 866.6120