多発性骨髄腫 - 形質細胞分離

米国ガイドラインについて

絵葉・ジャーディ
フィールド　アプリケーション　スペシャリスト

2022-08-01

STEMCELL™
TECHNOLOGIES
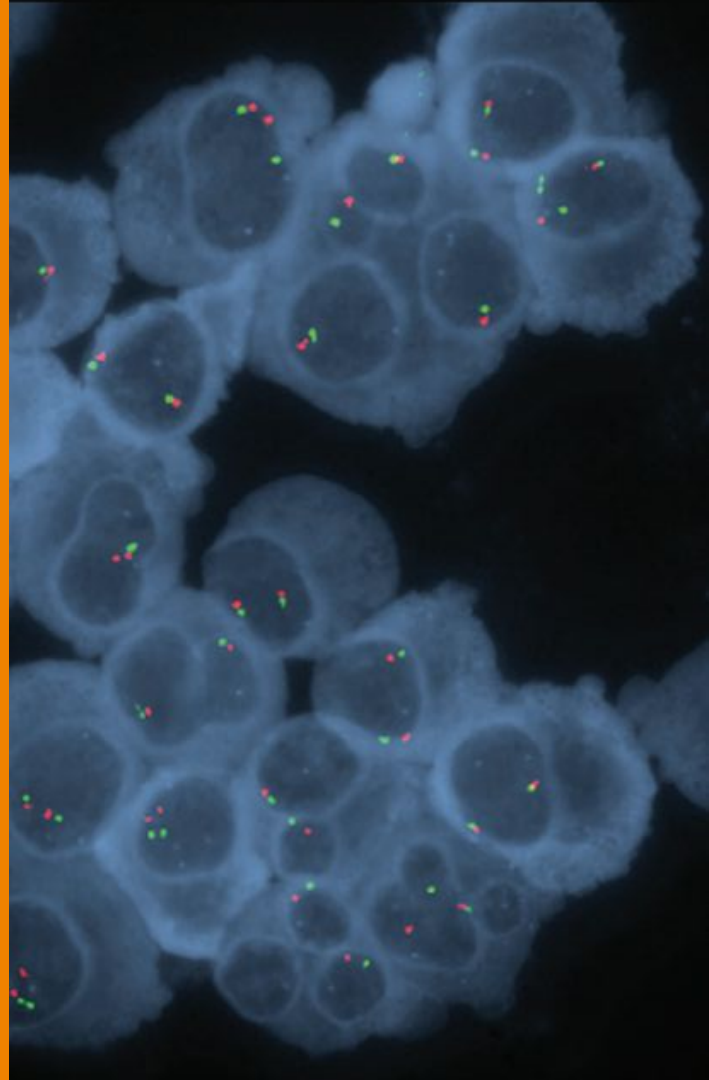
背景: 精製 CD138 形質細胞による多発性骨髄腫検査感度の向上

- 多発性骨髄腫の骨髄サンプルには非悪性細胞と悪性細胞が混在しています

- 非悪性細胞が大量に存在するサンプルの解析では、少量の悪性骨髄腫細胞検出が難しい場合があります

- CD138のポジティブセレクションで形質細胞を精製し、分析サンプル中の悪性骨髄腫細胞の割合を高めることにより、サンプルにおけるゲノム異常の検出が容易となります

- 多発性骨髄腫検査のための蛍光in situ ハイブリダイゼーション(FISH)、 マイクロアレイベースアッセイ、ゲノムシーケンス、遺伝子発現プロファイリングなどの後続の解析における感度を高めるために、米国では骨髄穿刺での採取後に形質細胞を濃縮することを推奨しています

技術資料PDF
無料ダウンロード

CD138+ 形質細胞分離 → FISH法検査 ワークフロー

患者様からの
骨髄サンプル

CD138+形質細胞を濃縮

細胞を固定

FISH法検査・解析

患者様への情報提供

Whole Bone Marrow | RoboSep™-enriched (CD138+)

CD138
Nuclei

Photo Courtesy of Greg Ahmann, Mayo Clinic

RoboSep™-S

分離前

分離後

3
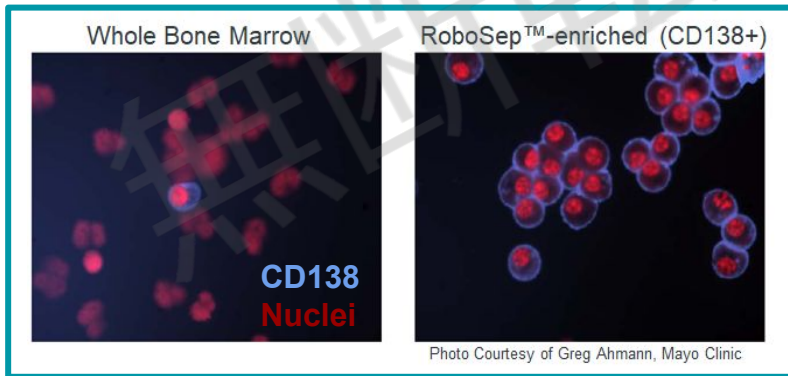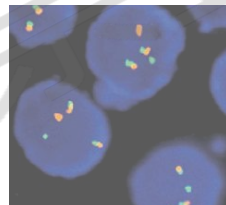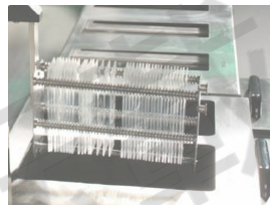
米国ガイドライン

- **NCCN Guidelines (Version 5.2022) for Multiple Myeloma**
  - National Comprehensive Cancer Network → 米国にある31軒のがんセンターの同盟
  - *"CD138 positive selected sample is strongly recommended for optimized yield"* for plasma cell FISH on bone marrow sample

The National Comprehensive Cancer Network recommends plasma cell enrichment for multiple myeloma FISH

- **American College of Medical Genetics Standards and Guidelines for Clinical Genetics Laboratories (2021 Revision, Section E)**
  - *"A bone marrow specimen is required for Multiple Myeloma. For FISH and/or CMA analyses, plasma cell separation is recommended to enrich for the CD138+ plasma cell fraction in bone marrow samples with low plasma cell percentages"*

米国の現状

- **Cancer Genomics Consortium Report (2018)**
  - Cancer Genomics Consortium → 臨床細胞遺伝学者、分子遺伝学者、および分子病理学者のグループ
  - *"To assess the current state of clinical molecular testing for myeloma, a 9-question survey of the Cancer Genomics Consortium membership and the American Cytogenetics Forum List was conducted from March to April 2017. There were 66 responses from respondents who self-identified as a cytogeneticist, molecular geneticist or pathologist (91%), laboratory technician (6%), or laboratory supervisor (3%)."*
  - *"Plasma cell enrichment prior to testing is widespread with > 85% of labs using CD138 + cell enrichment by a magnetic bead system (49% RoboSep-S, 29% Miltenyi, 10% EasySep, 3% epiSep). Laboratories that never use plasma cell enrichment were in the minority (15%) and several such groups stated the intention to establish enrichment as a standard protocol."*

Cancer Genomics Consortium Report

Assessing Genome-wide Copy Number Aberrations and Copy-Neutral Loss-of-Heterozygosity as Best Practice: An Evidence-Based Review from the Cancer Genomics Consortium Working Group for Plasma Cell Disorders

Trevor J. Pugh, James M. Fink, Xinyan Lu, Susan Mathew, Joyce Murata-Collins, Pascale Willem, Min Fang, on behalf of the Cancer Genomics Consortium Plasma Cell Disorders Working Group

PII: S2210-7762(18)30076-0
DOI: https://doi.org/10.1016/j.cancergen.2018.07.002
Reference: CGEN 8480

To appear in: *Cancer Genetics*

Received date: 13 March 2018
Revised date: 16 July 2018
Accepted date: 30 July 2018

In a bone marrow aspirate, what is the tumour cell content required to trigger plasma cell enrichment for array or FISH analysis? (n=66)		
<100% - we always perform enrichment	48	73%
<50%	1	1.5%
<40%	1	1.5%
<30%	0	0%
<20%	5	8%
<10%	0	0%
<5%	1	1.5%
0% - we never perform enrichment	10	15%
What methods do you use for plasma cell enrichment? (n=59)		
RoboSep-S magnetic bead purification	29	49%
Miltenyi magnetic bead purification	17	29%
EasySep	6	10%
EpiSep by Wavesense	2	3%

Enrichment performed by 85% of respondents

60% of respondents who perform enrichment use EasySep or RoboSep-S
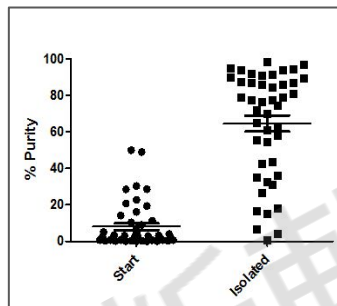
STEMCELL™ TECHNOLOGIES

CD138+ 形質細胞分離（多発性骨髄腫）

- 骨髄サンプル中のCD138陽性細胞の頻度は、疾患の進行度合いにより大きく異なる (0.1% → 60%)

- 少しでも濃縮することによって、FISH法検査の遺伝子異常の検出感度が上がる

RoboSep™-S

Multiple Myeloma FISH: Sample Data

Table 2. Linearity/AMR Verification

| Parameter	Upper Limit	Lower Limit
% CD138+ plasma cells in unsorted sample	60.8	0.29
Yield (CD138+ sorted plasma cell count)	3.3×10^6	8500
CD138+ absolute cell count in sorted fraction/μL	4752	25
% Recovery (yield/starting plasma cell count)	59.1	57.2
% Purity	99.7	41.2

Analytical Measurement Ranges (AMR) were defined for RoboSep sorter using serial dilutions of U266B1 plasma cell line spiked into CD Chex Plus control blood to obtain plasma cell concentrations ranging from <1% to 60% PCs. Duplicate 1 mL samples at each serial dilution were sorted on the RoboSep sorter. The unsorted and sorted fractions were analyzed by flow cytometry to determine absolute plasma cell counts and percent. Linear regression analysis of the dilution study gave an R value of 0.99 indicating the CD138 sort protocol was linear.

Table 1. FISH Results for Unsorted MM-Bone Marrow vs CD138+ Sorted Plasma Cells from the Same Bone Marrow

Sample	Sample Age (Days)	Sample Type	% of Cells Positive by FISH		
			13q Deletion	p53 Deletion	IgH Rearrangement
1	2	Unsorted	21	12	33
		Sorted	94	24	93
2	3	Unsorted	83	85	84
		Sorted	97	97	90
3	3	Unsorted	–	–	46
		Sorted	55[a]	–	52
4	3	Unsorted	–	–	25
		Sorted	94	–	87[b]
5	3	Unsorted	–	–	32
		Sorted	–	–	78[b]
6	3	Unsorted	–	–	39
		Sorted	–	12	39
7	5	Unsorted	22	–	28
		Sorted	91	–	93
8	5	Unsorted	18	–	32
		Sorted	14	–	93
9	6	Unsorted	23	–	34
		Sorted	92	10	99
10	6	Unsorted	23	29[c]	48
		Sorted	42	34[c]	84
11	7	Unsorted	23[d]	–	48
		Sorted	63[d]	–	84
12	7	Unsorted	–	–	26
		Sorted	8	–	15
13	8	Unsorted	–	–	29
		Sorted			90

Source: Targeted Cell-Sorting in Global FISH Studies: An Improved Method for Evaluating Multiple Myeloma. Marikar – Coplin et al. Quest Diagnostics Clinical Trials

STEMCELL™ TECHNOLOGIES

CD138+ 形質細胞分離（多発性骨髄腫）

Unseparated Cells (n=100)

Separated Cells (n=100)
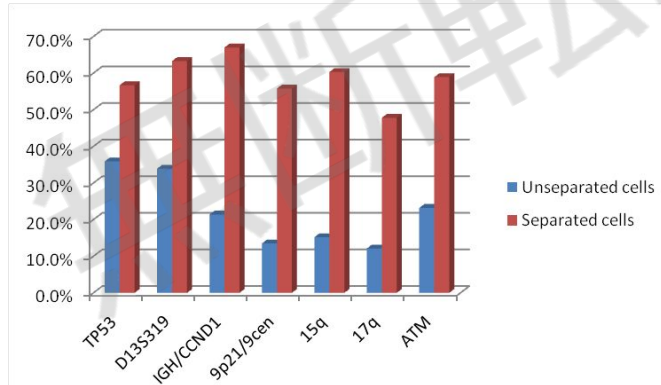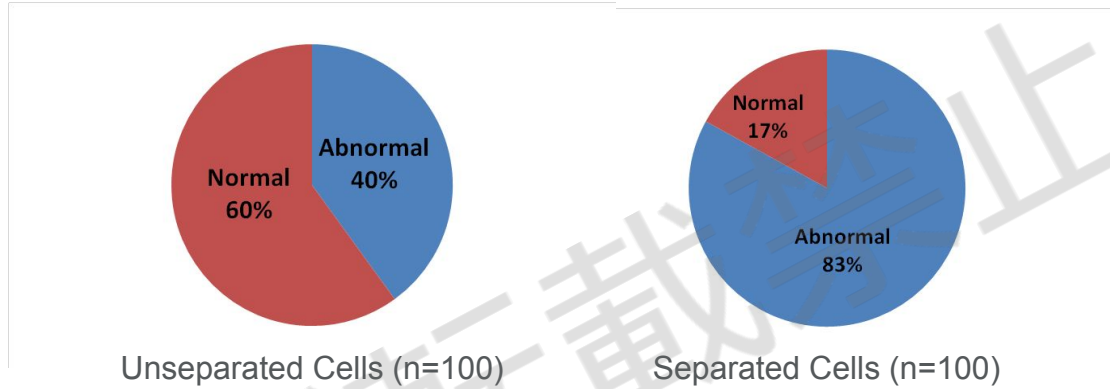
Increased detection of genetic aberrations in separated vs unseparated sample cohorts

製品紹介: EasySep™ CD138+ 形質細胞分離製品
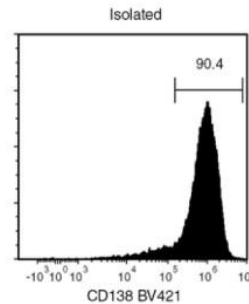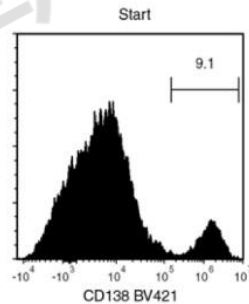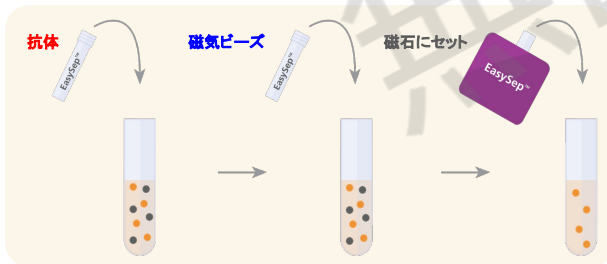

Silver Magnet　　　EasyEights™ Magnet　　　RoboSep™-S
（自動化装置）

17887 - EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit II

全血・骨髄 60mL

分離作業時間: 25 minutes

カラムフリー免疫磁気細胞分離

抗体　　磁気ビーズ　　磁石にセット

製品情報　　マニュアル

参考資料: オンデマンド ウェビナー

In hematologic cancers, including B cell lymphomas and multiple myeloma, malignant and non-malignant cells are mixed in the bone marrow or peripheral blood at variable frequencies. **Enrichment of lymphoid cells prior to further analysis using Fluorescence In Situ Hybridization (FISH) can enhance the sensitivity of this technique by increasing the presence of malignant cells.** This improves the resolution of FISH in detecting chromosomal abnormalities in patient samples, particularly in cases where disease burden is low. This webinar is sponsored by STEMCELL Technologies and Molecular at Abbott.

https://www.stemcell.com/technical-resources/educational-materials/videos-and-webinars/increasing-the-sensitivity-of-cytogenetic-analysis-of-hematologic-malignancies-through-cell-enrichment.html