PCST

Plasma Cell Screening Tube

Pacific Blue™	OC515	FITC	PE	PerCP-Cyanine 5.5	PE-Cyanine7	APC	APC-C750
CD45	CD138	CD38	CD56	β2micro	CD19	Cylgk	Cylgλ

Ref: CYT-PCST

CE

For In vitro Diagnostic Use

PCST VIALS ARE A LYOPHILIZED PRODUCT. PLEASE READ CAREFULLY THE FOLLOWING INSTRUCTIONS

FOR RECONSTITUTION:

The lyophilized PCST kit preserves the stability of the pre-mixed combination of antibodies. Reconstitute each lyophilized vial containing the pre-mixed combinations of cytoplasmic and surface antibodies with distilled water until the lyophilized pellet is totally dissolved.

For each surface staining vial reconstitution, it is necessary to use 180 µl of distilled water.

For each cytoplasmic staining vial reconstitution, it is necessary to use 70 µl of distilled water.

Once reconstituted, PCST kit is stable during one month from date of reconstitution if stored at 2-8º C protected from light.

INTENDED USE

Plasma Cell Screening Tube (PCST) is pre-mixed combination with 8 conjugated antibodies designed for identification and enumeration of plasma cells as well as the discrimination between normal polyclonal plasma cells such as in reactive plasmacytosis versus aberrant monoclonal plasma cells such as in monoclonal gammapathies of undetermined significance (MGUS), smoldering and symptomatic multiple myeloma (MM), plasma cell leukemias (PCL), and extramedullary plasmacytoma. In combination with the Euroflow LST and B-CLPD panels, this antibody panel will also contribute to the diagnosis of other plasma cell dyscrasias such as Waldenström´s macroglobulinemia and lymphoplasmacytic lymphoma (LPL) [1, 2].

SUMMARY AND EXPLANATION

Plasma cell disorders are a group of diseases most frequently characterized by the presence of clonal (neoplastic) plasma cells in the bone marrow capable of secreting a clonal Ig that can be detected in serum and/or urine. It includes different disease entities, among which multiple myeloma and monoclonal gammopathy of undetermined significance (MGUS) are the most prevalent and representative entities. Additionally, other less frequent clinical conditions associated with predominant extramedullary plasma cell locations and organ failure due to the accumulation of the clonal Ig (for example, amyloidosis) are also included in this group of diseases [3].

PCST kit recognizes by flow cytometry the antigens CD45, CD138, CD38, CD56, β2 microglobulin, CD19, Cylgκ and Cylgλ for the assessment and identification of aberrant and clonal plasma cells.

Flow Cytometry is a powerful tool for the analytical and quantitative characterization of cells that provides rapid, quantitative and multiparametric analysis of heterogeneous cell populations on a cell-by-cell basis. Flow cytometry is performed on cells in liquid suspension that have been incubated with fluorescently-labeled antibodies directed against specific cellular proteins. The relative fluorescence intensity of the positive cells indicates the amount of antibody bound to specific binding sites on the cells, and therefore provides a relative measure of antigen expression.

The use of 8 color panels in flow cytometry involves the use of new Cytognos fluorochromes here described:

- Orange Cytognos 515 is a fluorochrome excited with the violet laser (405 nm) and emits at 515 nm. This fluorochrome provides maximum resolution and narrow emission peaks, which results in little spectral overlap and minimal compensation requirements.
- APC-C750 is a tandem dye with a maximum emission peak at 779 nm, which grants bright signal, low unspecific noise and high photostability. When excited by light from a red laser, the APC fluorochrome can transfer energy to C750 molecule, which then emits at a longer wavelength. It is recommended to use a

780/60 nm band-pass filter along with a red sensitive detector to use in conjunction antibodies conjugated with APC and APC-C750.

PRINCIPLES OF THE PROCEDURE

Flow cytometry is an innovative technology by means of which different cell characteristics are simultaneously analyzed on a single cell basis. This is achieved by means of hydrodynamic focusing of cells that pass aligned one by one in front of a set of light detectors; at the same time they are illuminated by a laser beam. The interaction of the cells with the laser beam generates signals of two different kinds: those generated by dispersed light (FSC/SSC), which mainly reflects the size of the cell and its internal complexity, and those related to the emission of light by the fluorochromes present in the cell. These signals become electric impulses that are amplified and registered to be processed by a computer.
When the reagents are added to the sample, the mixture of fluorochrome-labeled antibodies present in the reagents bind specifically to the antigens they are directed against, allowing the detection by flow cytometry of the different lymphoid subsets.
The erythrocyte population, which could hinder the detection of the target population, is eliminated by the use of a red blood cell lysing solution previous to acquire the sample on the cytometer.
It is recommended follow the Calibration EuroFlow Standard Operating Protocol for Cytometer Setup. You will find a complete guide (Cytometer Setup SOP) on the web site www.euroflow.org, which includes recommendations for fix instrument configuration, FSC and SSC setting, target channel PMT setting, compensation setting and instrument performance monitoring[4,5]

REAGENT COMPOSITION

PCST kit contains sufficient volume for 25 tests distributed in lyophilized vials of 5 tests each. PCST kit includes a combination of Surface Membrane (Sm) and Cytoplasmic (Cy) staining antibodies identified as follows:
5 vials of 5 tests each for surface staining with the following lyophilized pre-mixed combination of antibodies:

- Anti human CD45-Pacific Blue™ antibody, clone: GA90, isotype: IgG2a.
- Anti human CD19-PE-Cyanine7 antibody, clone: 19-1, isotype: IgG1.
- Anti human CD138-OC515, clone: B-A38, isotype: IgG1
- Anti human CD38-FITC, clone: LD38, Isotype: IgG1
- Anti human CD56-PE, clone: C5.9, isotype: IgG2b
- Anti human β2 micro-PerCPCy5.5, clone: B2-1, isotype: IgG2a.

5 vials of 5 tests each for cytoplasmic staining with the following lyophilized pre-mixed combination of antibodies:

- Anti human CyIgκ-APC antibody, Polyclonal
- Anti human CyIgλ-APC-C750 antibody, Polyclonal

After reconstitution, components are supplied in phosphate-buffered saline (PBS) with 0,09% (m/v) sodium azide. Reagents are not considered sterile.

Fluorochrome	Pacific Blue™	OC515	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750
Marker	**CD45**	**CD138**	**CD38**	**CD56**	**β2 micro**	**CD19**	**CyIgκ**	**CyIgλ**
Clone	GA90	B-A38	LD38	C5.9	B2-1	19-1	Polyclonal	Polyclonal
Isotype	IgG2a	IgG1	IgG1	IgG2b	IgG2a	IgG1		

- Additionally, CD45-OC515, CD19-PE-Cyanine7 and SmIgλ-APC-C750 vials of 5 tests are included for compensation purposes. Compensation requirements for OC515 and APC-C750 are similar to Pacific Orange™ and APC-H7 respectively. Cytognos S.L. recommends adding 5 µl /test for the compensation purposes.

STORAGE CONDITIONS

PCST kit is stable until the expiration date shown on the label, when stored at 2-8º C. The expiration date applies to the lyophilized product. After reconstitution, vials with the pre-mixed combination are stable for one month when stored at 2-8º protected from light. Components should not be frozen or exposed to direct light during storage or during incubation with cells. Keep vials dry. Once opened, vials must be stored in a vertical position to avoid any possible spillage.
The expiration date of the reagent was determined by stability assays during use.

RECONSTITUTION

The lyophilized PCST kit preserves the stability of the pre-mixed cocktail of antibodies. Reconstitute each lyophilized vial containing the pre-mixed cocktail of cytoplasmic and surface antibodies with distilled water.

- **For surface staining vial reconstitution, it will be necessary to use 180 µl.**
- **For cytoplasmic staining vial reconstitution, it will be necessary to use 70 µl.**
- Unused volume of reconstituted vial is stable during one month from date of reconstitution if it is stored at 2-8º C.

WARNINGS AND RECOMMENDATIONS

1. For *in vitro* diagnostic use.
2. The kit is supplied ready to use. If it is altered by dilution or addition of other components, such conditions must be validated by the user.
3. The kit is stable until the expiration date shown on the label if it is properly stored. Do not use after the expiration date shown on the label. If the reagents are stored in conditions different from those recommended, such conditions must be validated by the user.
4. Alteration in the appearance of the reagents, such as the precipitation or discoloration indicates instability or deterioration. In such cases, the reagents should not be used.
5. It contains 0,09% (m/v) sodium azide (CAS-No. 26628-22-8) as a preservative, but even so care should be taken to avoid microbial contamination of reagent or incorrect results may occur.
   Indication(s) of danger:

H302	Harmful if swallowed

   Safety advice:

P264	Wash thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P301+P312	If swallowed, call a poison center or doctor/physician if you feel unwell.
P301+P330	If swallowed, rinse mouth.
P501	Dispose of contents/container in accordance with local/regional/national/international regulation.

6. All patient specimens and materials with which they come into contact are considered biohazards and should be handled as if capable of transmitting infection [6], and disposed according to the legal precautions established for this type of product. Also recommended is handling of the product with appropriate protective gloves and clothing, and its use by personnel sufficiently qualified for the procedures described. Avoid contact of samples with skin and mucous membranes. After contact with skin, wash immediately with plenty of water.
7. Use of the reagents with incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.
8. Any serious incident relating to the product must be reported to Cytognos S.L. as well as the competent professional authority of the Member State in which the user is established.

PROCEDURE

Material included

PCST kit is sufficient for 25 determinations, it includes the following reagents:

- **5 vials** of 5 tests each for **surface staining** (pre-mixed cocktail of CD45 Pacific Blue™/CD19-PE-Cyanine7/CD138-OC515/CD38-FITC/CD56-PE/β2 micro-PerCP-Cyanine5.5).
- **5 vials** of 5 tests each for **cytoplasmic staining** (pre-mixed cocktail of CyIgκ-APC / CyIgλ-APC-C750),
- Fix&Perm™ kit (Nordic-MUbio BV, The Netherlands).
- Additionally, CD45-OC515, CD19-PE-Cyanine7 and SmIgλ-APC-C750 vials of 5 tests are included for compensation purposes. Compensation requirements for OC515 and APC-C750 are similar to Pacific Orange™ and APC-H7 respectively.

Material required but not included

- Flow cytometer equipped with 405 nm violet laser, 488 nm ion argon laser, 633 red laser, 780/60 nm band-pass filter, and appropriate computer hardware and software associated.
- Test tubes suitable for obtaining samples in the flow cytometer used. Usually tubes with a rounded bottom for 6 ml, 12x75 mm are used.
- Automatic pipette (100 µl) and tips.
- Micropipette with tips.
- Vortex Mixer.
- Chronometer.
- Centrifuge.
- Pasteur pipette or vacuum system.

- Erythrocyte lysing solution.
- Wash buffer as phosphate buffered saline (PBS) + 0,09% of NaN_3 + 0,5% of Bovine Serum Albumin (BSA).

Preparation

Sample must be taken aseptically in a sterilized tube containing an appropriate anticoagulant (use of EDTA is recommended). Store the samples at 18-22ºC until they are to be tested. It is advisable to test within the 24 hours after their extraction. Hemolyzed samples or samples with suspended cell aggregates should be rejected. After the vial reconstitution, we recommend spin down carefully all vials before use to drive down any volume dislodged from the bottom of the tube.

1. Pipette 50 µl of sample to each tube and add 30 µl of the Surface staining vial (pre-mixed cocktail of CD45 Pacific Blue ™/CD19-PE-Cyanine7/CD138-OC515/CD38-FITC/CD56-PE/β2 micro-PerCPCy5.5). Add the necessary volume of PBS + 0,09% of NaN_3 + 0,5% BSA to reach a volume of 100 µl per tube.
2. Mix well.
3. Incubate for 30 min at room temperature (RT) protected from light.
4. Add 2 ml of PBS + 0,09% of NaN_3 + 0,5% of BSA to the cell pellet.
5. Mix well.
6. Centrifuge for 5 min at 540 x g.
7. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 50 µl residual volume.
8. Resuspend the cell pellet by mixing gently.
9. Add 100 µl of Reagent A (fixative solution; Fix&Perm™, Nordic-MUbio BV, The Netherlands).
10. Incubate for 15 min at RT protected from light.
11. Add 2 ml of PBS + 0,09% of NaN_3 + 0,5% of BSA to the cell pellet.
12. Mix well.
13. Centrifuge for 5 min at 540 x g.
14. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 50 µl residual volume.
15. Resuspend the cell pellet by mixing gently.
16. Add 100 µl of Reagent B (permeabilizing solution; Fix&Perm™, Nordic-MUbio BV, The Netherlands).
17. Mix well.
18. Add 10 µl of the intracellular antibodies pre-mixed vial (CyIgκ-APC / CyIgλ-APC-C750).
19. Mix well.
20. Incubate for 15 min at RT protected from light.
21. Add 2 ml of PBS + 0,09% of NaN_3 + 0,5% of BSA to the cell pellet.
22. Mix well.
23. Centrifuge for 5 min at 540 x g.
24. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 50 µl residual volume.
25. Resuspend the cell pellet in 200 µl PBS + 0,5% of BSA (without NaN_3).
26. Acquire the cells after staining or (if not immediately acquired) store at 4ºC maximally for 1 hour until measured in the flow cytometer.

It is recommended follow the Calibration EuroFlow Standard Operating Protocol for Cytometer Setup. You will find a complete guide (Cytometer Setup SOP) on the web site www.euroflow.org, which includes recommendations for fix instrument configuration, FSC and SSC setting, target channel PMT setting, compensation setting and instrument performance monitoring.
Appropriate compensation settings are required for the acquisition of this tube. Most fluorochromes emit also in surrounding inappropriate channels but this spillover can be mathematically corrected. Single stained tubes are used for compensation settings and for this purpose a sample of CD45-OC515, CD19-PE-Cyanine7 and other of SmIgλ-APC-C750 are included in the kit.

Flow Cytometry Analysis

Cytognos recommends the use of the **analysis software Infinicyt™**, which is capable to use pattern recognition and store analysis strategies to apply in batch to other samples using always the same criteria. You will find complete information about Infinicyt™ on the web site: www.infinicyt.com.

ANALYTICAL EFFICIENCY

Specificity:

-CD38 and CD138 antigens were selected for efficient identification of plasma cells and to distinguish between normal/reactive and clonal plasma cell compartments based on their most frequent aberrant phenotypes.
-β2-microgloblulin antigen recognizes the β chain which non-covalently associates with the α chain to form the HLA Class I antigen complex. It has also been shown that the plasma cell surface expression of β2

microglobulin negatively correlates with its serum levels and a better outcome, become an additional potentially attractive prognostic marker.
-CD19 antigen is expressed on the cell surface of normal and neoplastic B cells, and it is not expressed by T cells, monocytes and granulocytes.
-CD56 antigen is expressed on all natural killer cells (activated and resting) in human peripheral blood and also in a CD3+ T cell subset.
-Cytoplasmic anti-Kappa Light Chains react with free kappa light chains as well as intact immunoglobulin molecules bearing kappa light chains.
-Cytoplasmic anti-Lambda Light Chains react with free lambda light chains as well as intact immunoglobulin molecules bearing lambda light chains.
-CD45 antigen recognizes human leukocytes including lymphocytes, monocytes, granulocytes, and eosinophils. Erythrocytes, platelets and non-hematopoietic cells do not express CD45 antigen.

Expected Values

Each laboratory should establish its own normal reference ranges for lymphocyte subset counting, since such values may be influenced by age, sex and race. The reference ranges for the different subsets shown in the following table are expressed as the percentage from the reference populations. Data correspond to n = 10 multiple myeloma bone marrow samples acquired in a BD FACSCanto II cytometer and analyzed using Infinicyt™ software (Cytognos SL, Salamanca, Spain).

Cell population	Reference population	Mean (%) ± SD (range)	CV (%)
Lymphocytes	leucocytes	11.31 ± 5.48 (5.26 - 21.49)	48.48
B-cells	lymphocytes	19.86 ± 15.01 (0.58 - 49.25)	75.57
Kappa- B cells	B-cells	44.42 ± 16.67 (12.72 - 63.67)	37.52
Lambda-B cells	B-cells	37.17 ± 13.68 (11.38 - 63.64)	36.81
NK cells	lymphocytes	3.15 ± 2.64 (0.51 – 9.62)	83.6

The following table shows the expected Mean Fluorescence Intensity (MFI) of the different antibodies included in this PCST kit regarding target population. Data correspond to n=10 multiple myeloma bone marrow samples acquired in a BD FACSCanto II cytometer and analyzed using Infinicyt™ software (Cytognos SL, Salamanca, Spain).

Antibody	Fluorochrome	Cell population	Average MFI ± SD (Range)	CV (%)
CD45	Pacific BlueTM	CD45+/SSCLow Lymphs	22371.18 ± 9611.37 (4384.24 – 41720.50)	42.96
CD138	OC515	Plasma cells	2375.27 ± 1474.74 (705.72 – 6366.52)	62.09
CD38	FITC	Plasma cells	13773.08 ± 10319.73 (1716.82 – 36305.62)	74.93
CD56	PE	CD56+/CD3-/CD19- NK cells	2564.02 ± 962.82 (919.12 – 4442.38)	37.55
β2microglobulin	PerCP-Cyanine5.5	β2m+/CD45+/SSCLow Lymphs	42419.22 ± 15880.95 (11031.63 - 66154.40)	37.44
CD19	PE-Cyanine7	CD19+ B cells	8326.01 ± 2202.14 (3842.45-11524.31)	26.45
cyLAMBDA	APC C750	CD19+ B cells	11707.30 ± 35036.82 (4148.39 – 30785.24)	68.62
cyKAPPA	APC	CD19+ B cells	35408.78 ± 8033.45 (2937.65 – 107872.55)	98.95

Accuracy

Main lymphocyte subset percentages obtained with Cytognos PCST screening tube were compared with results obtained with the reference combination proposed by EuroFlow consortium [Van Dongen et al. Leukemia (2012) 26: 1908-1975]. The comparison of n= 10 samples with both methods shows that PCST is equivalent. Data were analysed with Infinicyt™ software and the following table indicates that the results are substantially equivalent in their reactivity on multiple myeloma samples in terms of percentage of the different lymphoid subsets. Data were analysed with Infinicyt™ software.

Cell population	Reference population	Control Method Mean (MFI) ± SD (range)	Cytognos PCST Mean (MFI) ± SD (range)	Mean differences (%)	CV Control (%)	CV Cytognos (%)	p-value
CD45	CD45+/SSCLow Lymphs	9894.93 ± 1719.47 (6578.72 – 12053.27)	22371.18 ± 9611.37 (4384.24 – 41720.50)	126.09	17.38	42.96	0.000
CD138	Plasma cells	2154.10 ± 1445.25 (500.62 – 6366.52)	2375.27 ± 1474.74 (705.72-6366.52)	10.27	67.09	62.09	0.83
CD38	Plasma cells	16928.38 ± 11032.57 (1672.12 - 37856.57)	13773.08 ± 10319.73 (1716.82-36305.62)	18.64	65.17	74.93	0.005
CD56	CD56+/CD3-/CD19- NK cells	2409.13 ± 632.46 (1544.82-3279.42)	2564.02 ± 962.82 (919.12-4442.38)	6.43	26.25	37.55	0.33
β2 microglobulin	β2m+/CD45+/SSCLow Lymphs	19124.88 ± 7279.99 (10158.59-30098.74)	42419.22 ± 15880.95 (11031.63-15880.95)	121.80	38.07	37.44	0.000
CD19	CD19+ B cells	9658.61 ± 2174.34 (7025.29-13715.10)	8326.01 ± 2202.14 (3842.45-11524.31)	13.80	22.51	26.45	0.001
cyLAMBDA	CD19+ B cells	3518.94 ± 3062.26 (657.95-11472.50)	11707.30 ± 8033.45 (4148.39-30785.24)	232.69	87.02	68.62	0.01
cyKAPPA	CD19+ B cells	14202.30 ± 9327.76 (2446.33-25766.41)	35408.78 ± 35036.82 (2937.65-107872.55)	149.32	65.68	98.95	0.002

Repeatability

Ten different multiple myeloma samples were stained with 2 different lots of PCST screening tube were assessed. Each pair of data was analyzed to evaluate MFI differences of the different antibodies included in this PCST kit. Data were analyzed with Infinicyt™ software.

Antibody	Fluorochrome	Cell population	Lot	Average MFI	% MFI differences	SD	CV (%)	p-value
CD45	Pacific BlueTM	CD45+/SSCLow Lymphs	1	30003.96	103.58	5774.05	19.24	0.000
			2	14738.40		5675.95	38.51	
CD138	OC515	Plasma cells	1	2167.55	17.74	1070.81	49.40	0.48
			2	2634.92		1915.43	72.69	
CD38	FITC	Plasma cells	1	11055.50	35.61	7788.51	70.45	0.003
			2	17170.06		12526.14	72.95	
CD56	PE	CD56+/CD3-/CD19- NK cells	1	2976.37	38.33	843.49	28.34	0.06
			2	2151.67		935.88	43.50	
β2 microglobulin	PerCP-Cyanine5.5	β2m+/CD45+/SSCLow Lymphs	1	49974.06	43.34	13239.10	26.49	0.004
			2	34864.38		15176.42	43.53	
CD19	PE-Cyanine7	CD19+ B cells	1	8696.96	9.33	2196.34	25.25	0.05
			2	7955.05		2260.07	28.41	
cyLAMBDA	APC C750	CD19+ B cells	1	6576.02	60.95	1772.72	26.96	0.02
			2	16838.58		8697.44	51.65	
cyKAPPA	APC	CD19+ B cells	1	16457.50	70.99	12105.17	73.55	0.007
			1	56728.96		40708.41	71.76	

LIMITATIONS

- Samples should be stored at 18-22ºC and be tested within the 24 hours after they are obtained.
- It is advisable to acquire stained samples on the cytometer as soon as possible to optimize the results. Non viable cells may stain nonspecifically. Prolonged exposure of whole blood samples to lytic reagents may cause white cell destruction and loss of cells from the target population.
- When using whole blood procedures, all red blood cells may not lyse under following conditions: nucleated red blood cells, abnormal protein concentration or hemoglobinopathies. This may cause falsely decreased results due to unlysed red blood cells being counted as leucocytes.
- Results obtained by flow cytometry may be erroneous if the cytometer laser is misaligned or the gates are improperly set.
- Each laboratory should establish a normal range for blast cells using its own test conditions. We recommend follow the EuroFlow antibody panels [4, 5] together with the EuroFlow instrument set-up, sample preparation and data analysis procedures [5].

- Certain patients may present special problems due to altered or very low number of certain cellular population.
- Cells separated from whole blood by means of density gradients may not have the same relative concentrations of cells as unseparated blood. This may be relatively insignificant for samples from individuals with normal white blood cell counts. In leucopenic patients, the selective loss of specific subsets may affect the accuracy of the determination.
- It is important to understand the normal pattern of expression of these antigens and its relation to the expression of other relevant antigens to carry out an adequate analysis.
- Abnormal states of health are not always represented by abnormal percentages of certain leucocyte populations. An individual who may be in an abnormal state of health may show the same leucocyte percentages as a healthy person. For this reason, it is advisable to use the test results in combination with other clinical and diagnosis data.

QUALITY CONTROL

- To obtain optimum results it is advisable to verify the precision of pipettes and that the cytometer is correctly calibrated.
- It is recommended follow the Calibration EuroFlow Standard Operating Protocol for Cytometer Setup. You will find a complete guide (Cytometer Setup SOP) on the web site www.euroflow.org, which includes recommendations for fix instrument configuration, FSC and SSC setting, target channel PMT setting, compensation setting and instrument performance monitoring.
- To evaluate the non-specific binding of the reagent, an appropriated isotype control tube can be prepared.
- This product has been manufactured in accordance with standards of production and quality system of the ISO 13485:2012 standard.

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WARRANTY

This product is warranted only to conform to the quantity and contents stated on the label. There are no warranties that extend beyond the description on the label of the product. Cytognos's sole liability is limited to either replacement of the product or refund of the purchase price.

EXPLANATION OF SYMBOLS

	Use by (YYYY-MM)
	Storage temperature limitation
	Keep out of sunlight
	Consult instructions for use
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Manufacter

PRODUCED BY

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