PMM Product Summary 2021

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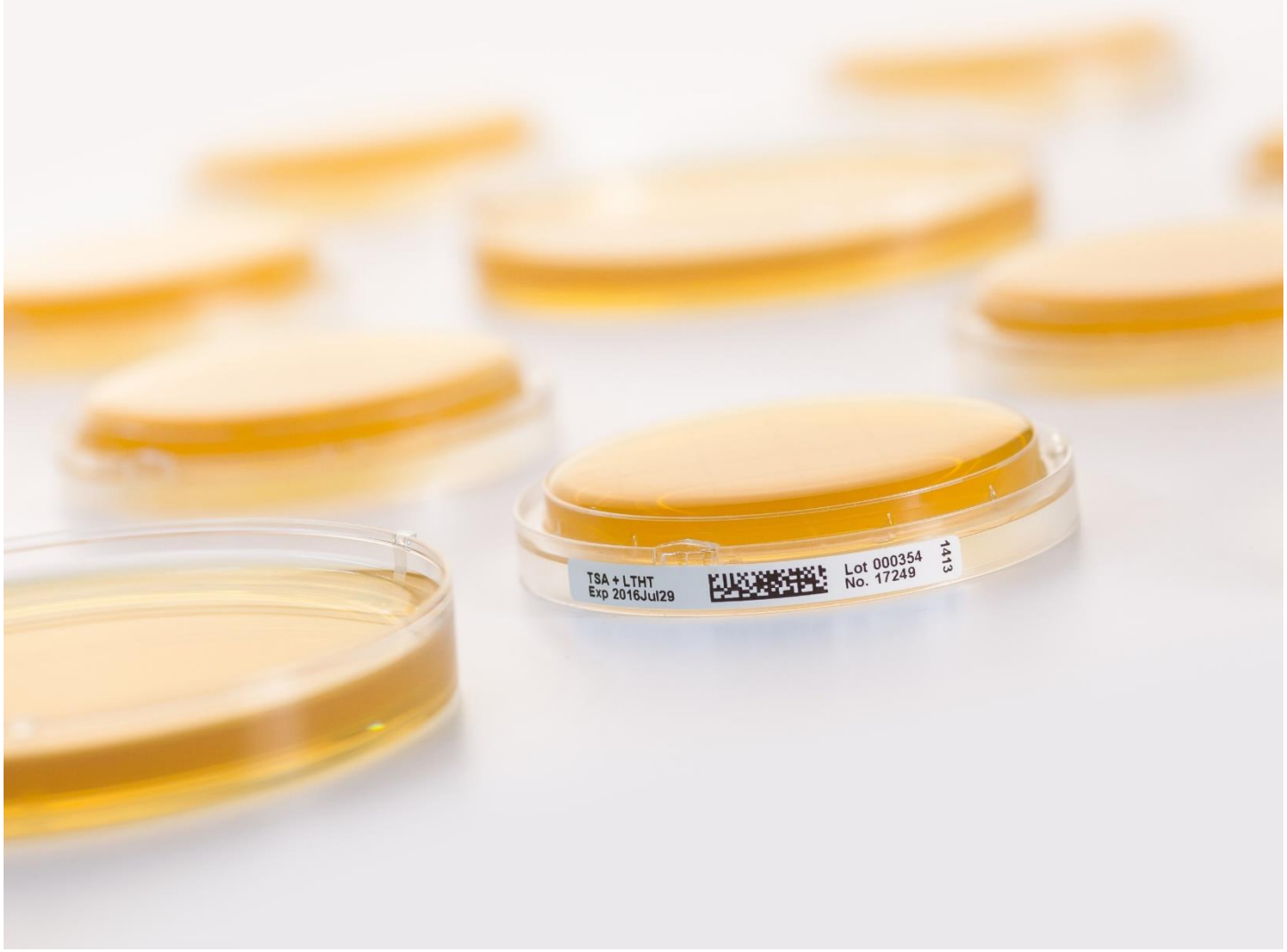

Ready-to-use Nutrient Media for pharmaceutical applications

CSG = Click & Safe Gamma Irradiated
For use in critical environments like Clean room class A and B as well as in isolators

CS = Click & Safe
For use in less critical environments like clean room class C and D

*If you have received one copy of this document, please ask for an update version if you would like to receive the latest version. There will be no automated update service.

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General company Information

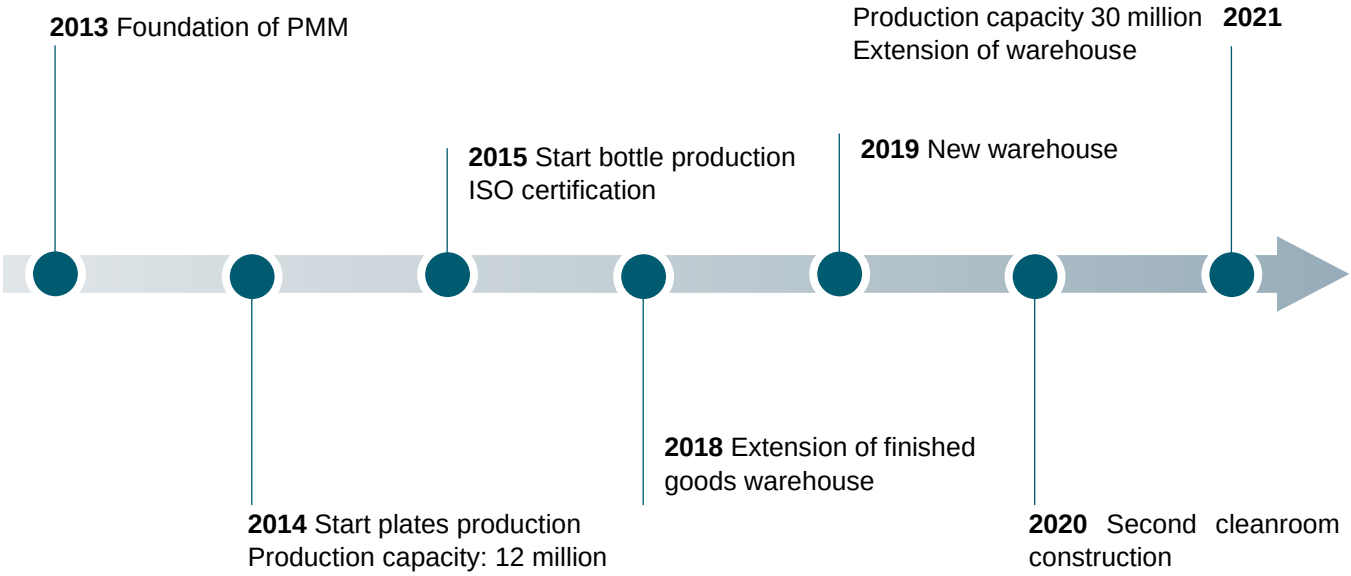
PMM is a leading high-quality producer of ready-to-use culture media. The company was founded January 2013 by Dr. Rolf Müller, who has already founded Heipha Dr. Müller GmbH in 1973.

As the focus of the company is exclusively the pharmaceutical and Medical devices applications, we are working aligned to the GMP-requirements expected by the pharmaceutical industry. The production is performed in clean rooms which are in accordance with the EC-GMP guidelines, ISO 14644 and FDA Guidance for Industry “Sterile Drug Produced by Aseptic Processing” with respect to air-exchange rates, laminar air-flow, clean-room classification and particle rates at rest. The quality of our work is led by established Quality Management System.

As we are not producing pharmaceutical drugs or active pharmaceutical ingredients, there is no authority in charge of approving our facility, our products or the way we manufacture. Therefore, we are regularly audited by our customers. In case you would like to audit us, please feel free to contact us any time.

We have been originally certified according **ISO 9001:2015** in November 2015, followed by the re-certification in 2018. The actual certificate is available for download at www.pmm-leimen.de

As 2021 begins, we are proud to announce the validation of a second cleanroom for the production of plated media, increasing our plates production capacity by 250%, bringing our facilities to 2 cleanrooms for the production of plates and one production unit of bottles. In conjunction with an extension of our warehousing capacity for packaging materials by more than 200%, it will help us to support our growth while helping to keep the quality of our products to the highest level possible.



BSE-/TSE- Policy

Beside chemically well-defined raw materials an essential part of nutrient media are complex mixtures of peptones or extracts of biological origin. To assure the use of safe raw materials, the animal source and its country of origin is reviewed for each new batch of raw material. A specific attention is given to the control of ruminant material which might pose the risk of transmitting animal spongiform encephalopathy agents.

In compliance with the “Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy via human or veterinary medicinal products” (EMA 410/01) we check the Certificate of Origin (CoO) of the raw material in respect to the specified animal source, the country of origin and the infectivity category.

We neither store or process ruminant raw materials obtained from high infectivity tissues (IA) nor ruminant raw materials whose animal source originates from countries or regions with an undetermined BSE risk (cat. C/GBR IV).

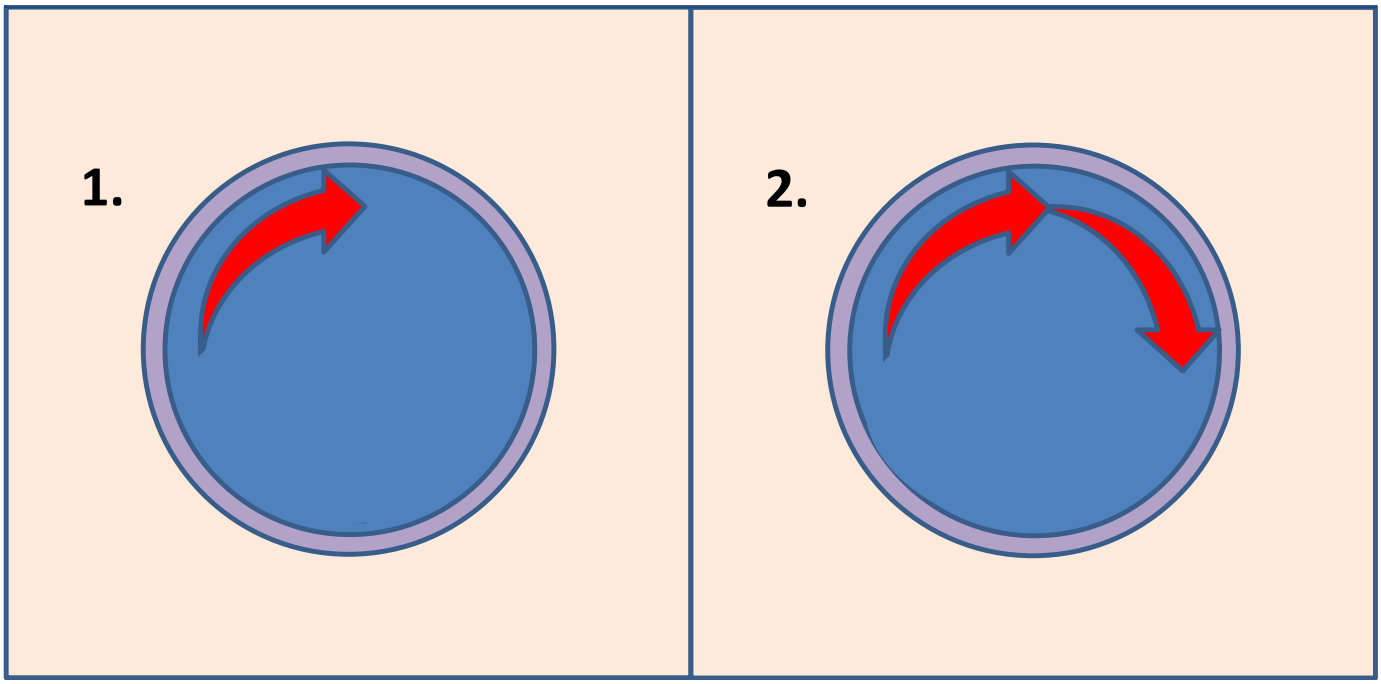
Locking Lids

90 mm as well as contact plates used by PMM have the newly designed locking lid functionality, offering the option to incubate the plates either in Vent- or alternatively in the Closed-position. All plates are delivered in the open position and can be turned to one of the locked positions after sampling.

For standard incubations of aerobic microorganisms, it is recommended to incubate the plates in in the Closed-Position. For incubating the plates in the Closed-Position, turn the lid clock-wise to the final stop-position, bypassing the first click-stop.

For the detection of anaerobic microorganisms, the plates have to be incubated in the Vent-Position in anaerobic conditions (e.g., in an anaerobic jar or in an anaerobic incubator). The Vent-Position allows an improved gas exchange with the incubation environment, thus optimizing the incubation conditions for the detection of anaerobic microorganisms. For this incubation, please turn the lid until the first click-stop (=Vent-Position).

1. Turn the lid clock-wise to the first click stop ➔Vent position
2. Turn the lid clock-wise to the final stop position ➔Closed position



This newly developed locking-lid has been designed to prevent the lid to be turned into the wrong direction, thus incubating the plates unintentionally in the Vent-Position.

Growth of anaerobic microorganisms

To evaluate the Vent position of PMM contact plates, the growth of eight anaerobic test strains under anaerobic conditions was tested and compared to the Closed position. As a result, all strains showed good growth results if the lid was locked into the Vent position, whereas growth was clearly reduced when incubating the plates in the Closed-position.

Closing the lid in the Vent position results in a small gap between the lid and the bottom of the plate, thus allowing a higher gas exchange rate. This is important if anaerobic isolates are incubated in an anaerobic jar, because this allows for an effective and fast removal of oxygen form the incubation environment.

Therefore, it is highly recommended to use the vent position for the incubation of anaerobic microorganisms and to really make sure, that the lids have been closed in the correct latch.

Incubation of plates

Plates for microbial tests have to be incubated according to the instructions for the specific application. For most of the applications in the pharmaceutical industry, the detailed incubation conditions are listed in the official guidelines, like EP or USP. However, media used for environmental monitoring are quite often a point of discussion in respect to the incubation conditions to be applied. Therefore, please find below our recommendations for the incubation conditions in case one plate is used for both, detection of total aerobic counts as well as detection of yeasts and molds:

Recommended incubation of environmental monitoring plates used for environmental monitoring

1. in clean room classes A, B, in isolators and RABS:

Incubate first at 20 to 25°C for at least 72 hours (e.g. 72 to 120 hours, followed by an incubation at 30 to 35°C for at least 48 hours (e.g. 48 to 72 hours).

2. In clean room classes C and D and in non-controlled areas:

Incubate first at 30 to 35°C for at least 48 hours (e.g. 48 to 72 hours), followed by an incubation at 20 to 25°C for at least 72 hours (e.g. 72 to 120 hours

The recommendation is based on the following considerations:

In clean room classes C and D molds could be isolated – if plates would be incubated at 20 to 25°C the growth of molds might overgrow colonies of bacteria, thus making a correct evaluation of total aerobic count almost impossible. Therefore, plates should be first incubated at the optimum conditions for bacteria, followed by the optimum incubation conditions of yeasts and molds.

In clean rooms class A and B, isolators as well as RABS, incubation should take place first at 20 to 25°C followed by an incubation at 30 to 35°C. Incubating at the lower temperature first will guarantee that temperature sensitive yeasts and molds, sensitive to temperatures above 30°C, can be detected as well.

Plates used for environmental monitoring of total aerobic count as well as for yeasts and molds should be incubated in the closed lid position. Furthermore, it is recommended to incubate the plates upside down (lid showing to the bottom).

Petri Dish

Sterile 90 mm and 55mm contact plates are made out of pure polystyrene. Plates are irradiated prior to the use in our production to minimize the risk to introduce a bioburden to our cleanroom as well as to minimize the risk of contamination of our finished products.

Whereas Petri dishes for most of our products are made from clear polystyrene, the plates for SDA media are made from slightly pink tinted polystyrene. The color allows an easy differentiation between TSA and SDA plates, thus avoiding mixing up plates during application and incubation.

Whereas the color is clearly visible when looking from the side onto a plate, it is only faintly visible when looking from the top onto the plate. Therefore, the evaluation of the tinted SDA plates is not affected by the color.

The color used to tint the polystyrene is made from an FDA approved, inert material, not migrating out of the plastic and not interacting with the media filled into the plates.

Desiccant

To minimize the amount of condensation in our products a desiccant bag is added. The desiccant bag is designed to pick up condensation which may be generated by production or transportation of the plates. The desiccant bags are gamma-sterilized prior to the application to the plate staples. The bag material is made from Tyvek®, the desiccant material itself is silica gel.

Multilayer foils used for packaging of CS and CSG products

All PMM culture media in plates are either single bagged or triple bagged in a multi-layer foil. The foil for all layers is identical. It is composed of a multilayer film with a special gas barrier layer. The different layers of the multi-layer foil are lined together by a solvent free co-extrusion process.

Multilayer foils are necessary to achieve the required strength of the welding seam as well as to protect the medium. The foil is especially designed to have a high gas barrier. Therefore, the media inside the bags are protected against dehydration and furthermore potentially harmful gas (e.g., H_2O_2) cannot penetrate easily from the outside to the inside.

The specifications of the foil we are using are as follows:

Oxygen permeability: <= 2,0 $cm^3/m^2*d*bar$ (at 23°C / 0% r.h.; measured acc. DIN 53380)

Water vapor permeability: <= 1,5 g/m^2*d (at 23°C / 85% r.h.; measured acc. DIN 53122)

Based on our experience the multilayer foil used for the packaging of PMM plates are perfectly suited to protect the plates against all commonly utilized sanitization methods used for introducing the products into critical environments like clean rooms and isolators.

Although the bag material is having high barrier characteristics for the permeability of gasses, a study completed by an external laboratory was performed, testing for the impermeability for VHP (Vaporized Hydrogen Peroxide). For this test, bags were introduced in an isolator and exposed to three succeeding decontamination cycles. Afterwards the H_2O_2- concentration inside the bags was measured.

In none of the original bagged samples hydrogen peroxide could be detected, proving that PMM plates packaging under the applied worst-case conditions is impermeable to H_2O_2.

The amount of foil required for packaging one staple of plates is as follows:

Contact plates: 28cm x 26,5cm = 742 cm^2 - less the sealing area of about 75cm^2 (approximately 667cm^2)

Sedimentation plates: 26cm x 30cm = 1080cm^2 - less the sealing area of about 125cm^2 (approximately 955cm^2)

Hydrogen peroxide permeability testing

For introducing media into an isolator or into RABS two main topics have to be considered:

1. Does the gassing cycle interfere with the quality of the product? and,
2. Does the medium inactivate hydrogen peroxide residues sufficiently when used in isolators having even a low residual VHP concentration?

Because media in isolators are exposed to hydrogen peroxide (H_2O_2) it is nowadays state of the art that media used in isolators must neutralize hydrogen peroxide. Acknowledging this it does not really make sense to perform a test on the tightness of the packaging material against hydrogen peroxide with plates themselves inactivating hydrogen peroxide. Therefore, it is mandatory to perform such test just for the packaging material.

In addition to the test for the impermeability of the packaging material, it is mandatory for all media to be used for active or passive air monitoring in isolators or RABS to use media being able to neutralize hydrogen peroxide.

Although the residual H_2O_2concentration in isolators is typically below 1ppm, this low concentration could result in false negative air monitoring results. Such false negative results are due to the large amount of air collected. During sampling of 1 m^3 of air containing a low hydrogen peroxide concentration almost all hydrogen peroxide is filtered out by the medium. This finally results in a situation, that the exhaust air of such active air monitoring shows a strongly reduced H_2O_2 concentration, whereas almost all of the H_2O_2out of the air is concentrated in the 30ml medium of the medium plate. This results in an accumulation of H_2O_2 in the medium in concentrations easily above 50 ppm H_2O_2, thus killing all potential contaminations, which finally could lead to false negative results.

Gamma-Irradiation

The irradiation of media is the last step of the media production process. Besides the production environment during the filling process the irradiation process is quite critical in order to provide the safety required for critical applications in the pharmaceutical industry. Although both gamma as well as beta-irradiation would be possible irradiation processes, mainly gamma-irradiation is used by media manufacturer for the production process of media. We at PMM are exclusively using gamma-irradiation for the irradiation of our plated media.

The irradiation doses need to be powerful enough to kill effectively the bio burden without influencing the quality of the medium. To guarantee the quality of gamma-irradiated media it is mandatory to control the quality of the raw materials very thoroughly.

PMM plates are irradiated at external irradiation service providers, which has been audited before. The media are packed in a certain loading scheme on a pallet and are irradiated always the same way. A dosimetry at each service provider has been performed, showing that at all locations on that pallet are within the required doses window of 9 to 20 kGy.

An indicator placed on each box of plates and on each multilayer foil confirm that the irradiation was successful.

For quality control testing plates which have been irradiated are used.

Insulated packaging material: silver colored air bubble foil

All PMM culture media in Petri dishes formats are packed in cardboard boxes with an additional insulating layer of silver-colored air bubble foil. The insulation is necessary to protect the plates against temperature fluctuations during transport and storage as well as to protect them from physical damages. This packaging makes shipment and storage of plates more economical. The temperature properties of the insulated material are in the same range or even better than Styrofoam boxes.

Contrary to the first impression, the coating is not made of aluminum. Instead of aluminum, the coating is made of polyester. Please find below the specifications of the air bubble foil:

- Material air bubble foil: HDPE = High Density Poly Ethylene
- Coating air bubble foil: Polyester
- Total weight per box: ca. 100 g (ca. 1 g per contact plate and 1.65 g per settle plate)

In addition to the space reduction, the new packaging offers ecological advantages compared to Styrofoam packaging. Instead of using about 2500 kg of Styrofoam for the packaging of 1 million plates with the new packaging only 1000 kg HDPE is required, thus saving roughly 60% of natural resources and impacting the cost of transport.

International Transport

For international transports outside of Europe we are using in addition to the “silver coated air bubble foil” additional thermal protection of the pallets shipped. The following options exist:

- Pallet-sized silver-coated air bubble foil (specification see above)
- Pallet-sized silver coated air bubble foil plus “water blankets”
- rPE coated Thermoliners (rPE = recycled PE)

Packaging scheme of PMM plates

PMM's packaging material are chosen to provide optimum space consumption for transport and storage. 11 boxes fit per layer on a euro pallet. PMM products are shipped standard wise on one-way plastic pallets. However, even if the pallets have a base, they are not designed to be stored in a high rack warehouse without support-trays.

For the sake of a better insulation for air transportation, a different packaging scheme is used, where only 10 boxes can be packed per layer. Additionally, the pallet height is limited to 6 layers.

Shipment on Euro pallets (120x80cm)

layer	Max. # of boxes	Approx. weight	# of contact plates	# of settle plates	Approx. height (cm)
0	-	5 kg	-	-	15 cm
1	11	44 kg	1100	660	38 cm
2	22	85 kg	2200	1320	60 cm
3	33	120 kg	3300	1980	82 cm
4	44	155 kg	4400	2640	104 cm
5	55	190 kg	5500	3300	126 cm
6	66	225 kg	6600	3960	150 cm
7	77	260 kg	7700	4620	175 cm

Lot size

The lot size of most products depends on the vessels used for the preparation of the medium. Our largest vessels can handle about 500 L of medium, corresponding to the following maximum lot sizes:

- **Contact plates:** about 27000 contact plates (55mm)
- **Sedimentation plates:** about 16000 sedimentation plates (90mm)

Labelling information- Data matrix code – ECC 200

Product labels contain 4 sets of information as well as the 2-dimensional data matrix code and an additional internal number which is printed on the very right end of the label vertically.

Top left: Short version of the product name: for example, TSA + LTHT (art. code 200)
Bottom left: Expiry date: YYYYMMMDD (for clarity the month is mentioned as JAN, FEB, MAR...)
Top right: Lot number: composed of 6 digits
Bottom right: Unique identification number composed of 5 digits
Middle: Data matrix code
Very right: internal number (vertically)

Read-Out of data matrix code - example from above: 100 000123 00284 230919

The combination of the lot number and the unique identification number allow to have a specific identification of each single plate provided by PMM. The data matrix code may allow to identify each plate and to combine these with sampling specific data like place of sampling, operator, date, time, etc., as well as to the evaluation of the plate. By using an electronic system this could result in a paperless environmental monitoring process, eliminating the need of writing on plates as well as transferring data by hand into an electronic system.

In addition to time savings, this can help to avoid mistakes when copying the data. More important, it reduces the risk of contaminating plates by unnecessary handling steps like writing on plates.

The data matrix code is printed on a white or light-colored label on the side of the plate. The labels are optimal for having a good contrast required to read the codes easily with a bar code reader. The location on the side allows reading/identifying the plate even if the plate is stacked in a staple. However due to time and space limitations the information which can be coded in such bar code is limited.

The data matrix code is composed of 20 digits, which code for 4 different sets of data. The code does not contain any separation codes or even any information about the media manufacturer. Therefore, the structure of the code is absolutely fixed like shown below:

- Digits 1-3 Art.-code (no translation table required, as our art. codes contain only 3 digits)
- Digits 4-9 Lot-No
- Digits 10-14 Unique identification number
- Digits 15-20 Expiry Date (YYMMDD)

Important Remarks:

If for example lot-numbers or identification-numbers contain less than 6 digits the number is shown filled with zeros to the left side of the number: example: the lot number 123 is shown in the bar code as 000123.

Other suppliers of ready-to-use media may use the same data matrix code format. However, PMM lot numbers are < 100.000, whereas other suppliers using codes structured the same way are using lot numbers > 100.000. Therefore, plates of PMM can be easily identified.

Shelf-life data of standard products

For all our standard products, the shelf-life is indicated on the Certificates of Analysis (CoA). The shelf-life of these products is described by the expression “shelf-life”, whereas on CoAs of products, where the shelf-life confirmation is still ongoing, this is indicated by the expression “preliminary shelf-life”. On request we can provide statements to show the actual shelf-life tests performed for each product.

Shelf-life of media: how long can the medium be used?

Shelf-life test performed at PMM are typically started at least four weeks after the date printed on the product. This guarantees that the products can be used up to the last day of the printed date, even if the read-out of the medium is after the printed date. In case you have enquiries about the shelf-life of a specific product, please feel free to ask for a statement regarding the actual shelf-life tests performed.

Shelf-life of media for customized products: preliminary determination of date of expiry

The shelf-life of media is mainly influenced by two factors, the composition of the medium as well as the packaging of the medium. In respect to the composition, the nature of the raw materials (stable or highly instable) and the oxidability of ingredients are most critical. In respect to the packaging, the gas impermeability, especially for water vapor and for oxygen are crucial. By using a packaging material with a high barrier against water-vapor and against oxygen a much longer shelf-life can be achieved compared to plates packed in gas-permeable materials.

When producing a medium for the first time, a shelf-life will be given to such new product. The preliminary shelf-life assigned to a product is based on experiments made with similar products, reduced by about 1/3. Of course, such preliminary shelf-life will be confirmed by tests throughout the preliminary assigned shelf-life, proving that the product is performing well at all stages.

In case that we were observing differences with the assigned shelf-life, we will inform the users immediately and we will adapt accordingly the date of expiry for all future lots. However, in most cases in the past, we were able to prove with three consequent lots that the shelf-life could be even extended. Such extension of shelf-life will be handled in our Change Control process and our customers will be informed about the confirmed new date of expiry.

Quality control of culture media

Quality control of culture media is critical for the evaluation of the media itself as well as for the evaluation of the results obtained with the media. Quality control of ready to use media is performed as followed:

Measurement of pH:

For measuring of pH value on solid medium, a flat surface electrode should be used. pH measured with flat surface electrodes might differ between manufacturers. Therefore, always use the same electrode – and, preferably - use the same type of electrode as your media supplier. At PMM the following electrode is used:

- InLab Surface, art.no. 51343157, Mettler Toledo AG

Flat surface electrodes can also be used for liquid media with the exception of liquids with a low ionic strength, like washing buffers. In this case a different, specific electrode is recommended. At PMM the following electrode is used:

- InLab Cool, art. no. 51343174, Mettler Toledo AG

Growth Promotion Test:

Inoculum:

Self-prepared, deep frozen working suspensions, freshly prepared suspensions from overnight cultures as well as commercial ready-to-use suspensions can be used. Depending on the application, the choice of different types of suspension might give different results. The most robust inoculum is typically the fresh overnight culture, diluted in peptone buffer to meet the required CFU number.

Please be aware of the following characteristics of the strains:

- In case *Aspergillus brasiliensis* spore suspension is prepared by your own, Tween 80 should be added to the dilution buffer to avoid spore aggregates (1%)
- When using commercial ready-to-use suspensions, the concentration mentioned on the certificate is valid for non-selective media only. In case of testing a selective medium, only a qualitative result is required, as stated in the EP/USP. Thus, always compare the result to that of an already approved lot of the same medium.

In routine testing at PMM a self-prepared stable spore suspension of *Bacillus subtilis* instead of vegetative cells is used for the growth promotion test.

Inoculation:

At PMM inoculation is performed with a spiral plater, inoculating 20 - 100µl per plate, depending on strain suspension. In case no spiral plater is available, the following instructions should be followed:

- Spread the suspension on the surface and immediately streak the inoculum with a spatula.
- Streak for less than 5 seconds – and never to dryness. This is very important for Gram-negative test strains

Incubation of the plates should not be started before the complete suspension volume is diffused into the medium.

Temperature stress during storage

Optimum storage temperatures for media depend on the composition and packaging. Most media used in the pharmaceutical industry do contain quite stable ingredients. Therefore, such media could be stored in a temperature range between 2°C and 25°C without having any influence on the media quality. However, packaging of media may have an important influence on the quality of the medium. To date, there are mainly 2 different ways of packing media:

1. Using a gas-permeable packaging like Tyvek
2. Using a gas-impermeable packaging, e.g., a multi-layer foil

A gas-permeable packaging has the advantage that condensation could evaporate through the foil. At the same time this gas-permeability has the disadvantage that the media start to lose water as soon as they are produced. This drying-out limits the shelf-life of these products. Furthermore, the media have to be stored at low temperatures to minimize the drying-out effect, as the water vapor pressure is lower than at higher temperatures. However, due to the storage at low temperatures these media show quite often quite some condensation.

A gas-impermeable packaging has the advantage, that only very limited amount of water can evaporate, therefore the shelf-life of media packed this way is not limited by the drying-out effect described above. On the other hand, condensation once created cannot evaporate and therefore the creation of condensation has to be avoided. For this reason, media packed in gas-impermeable foil must be stored at stable temperature within the range of 15°C to 25°C.

PMM culture media are all packed in gas-impermeable foil and should be stored at 15°C to 25°C. We have performed worst-case storage studies where the plates (art. 100.0100 as well as 200.0060) were stored at 2°C-6°C for three days and then at 39°C to 41°C for additional three days. At the end of the shelf-life, the plates were tested according to our regular QC testing. All lots passed the tests.

Temperature stress study

The articles listed in the table below are representative selection of media manufactured at PMM. The media have been stressed exposing them at least 72 hours to a temperature of 2°C to 8°C as well as 72 hours to a temperature of 30°C to 35°C. after the temperature stress have been performed, the media have been stored at specified storage conditions (15°C to 25°C) for at least 30 day after the shelf-life stated and then the shelf-life tests were performed.

The shelf-life tests comprise all tests of a regular article specific quality control for release of a batch (please refer to CoA).

Plated media - stressed for at least 3 days at >30°C as well as 3 days at 2 to 8°C (regular storage at 15-25°C)

Item n°	Description	Shelf-life	Tests
100.0100	TSA-LTHT Cont. CSG	270 days	≥300 days
101.0100	TSA-U+ Cont. CSG	270 days	≥300 days
114.0100	TSA + LTG + Lac 2G Cont. SCG	240 days	≥270 days
116.0100	TSA + LT + β-Lac I/II Cont. CSG	240 days	≥270 days
120.0100	SDA + LTHT Cont. CSG	240 days	≥270 days
200.0060	TSA-LTHT 90mm CSG	270 days	≥300 days
210.0060	TSA 90mm CSG	270 days	≥300 days
214.0060	TSA + LTG + Lac 2G Cont. SCG	240 days	≥270 days
216.0060	TSA + LT + β-Lac I/II Cont. CSG	240 days	≥270 days
220.0060	SDA + LTHT Cont. CSG	240 days	≥270 days
300.0120	TSA-LT 55mm CS	270 days	≥300 days
400.0060	TSA-LT 90mm CS	270 days	≥300 days
410.0060	TSA 90mm CS	270 days	≥300 days
425.0060	SDA + CA 90mm CS	240 days	≥270 days
426.0060	SDA 90mm CS	240 days	≥270 days
450.0060	Cetrimide Agar 90mm CS	180 days	≥210 days
455.0060	Mannitol Salt Agar 90mm CS	180 days	≥210 days
460.0060	MacConkey Agar 90mm CS	180 days	≥210 days
465.0060	VRBD 90mm CS	180 days	≥210 days
470.0060	XLD Agar 90mm CS	120 days	≥150 days
490.0060	R2A 90mm CS	240 days	≥270 days

For all stressed media the results passed the defined article specifications without any problem and in comparison, with the results at the time of release of the respective batch, no significant differences could be observed.

As for the temperature stress test selected media are comprising the most sensitive media PMM produces, the results can be considered as a good indication that plated media produced by PMM do not suffer from temperature stress up to 72 hours.

Bottled Media - stressed for at least 3 days at >30°C (regular storage at 2-25°C)

Item n°	Description	Shelf-life	Tests
500.B100	TSB 100ml (screw cap bottle)	360 days	≥390 days
508.B090	TSB + Tween 80 (0,1%) 90ml (screw cap bottle)	360 days	≥390 days
520.S100	TSB 100ml (infusion bottle)	360 days	≥390 days
530.S100	FTM clear 100ml (infusion bottle)	360 days	≥390 days
540.S100	Fluid A 100ml (infusion bottle)	360 days	≥390 days
542.D000	Fluid A 1000ml (screw cap bottle)	360 days	≥390 days
552.D000	Fluid D 1000ml (screw cap bottle)	360 days	≥390 days
566.B090	NPB + Tween 80 (0,1%) 90ml (screw cap bottle)	360 days	≥390 days
571.B090	NPB 90ml (screw cap bottle)	360 days	≥390 days
572.B090	NPB + LTH 90ml (screw cap bottle)	360 days	≥390 days
573.B200	NPB + Tween 80 (0,1%) 200ml (screw cap bottle)	360 days	≥390 days
579.B090	NPB + Tween 80 (3%) 90ml (screw cap bottle)	360 days	≥390 days
580.B100	SDB 100ml (screw cap bottle)	360 days	≥390 days
581.B100	MacConkey Bouillon 100ml (screw cap bottle)	270 days	≥300 days
600.B200	TSA 200ml (screw cap bottle)	360 days	≥390 days
601.B200	SDA 200ml (screw cap bottle)	360 days	≥390 days

For all stressed media the results passed the defined article specifications without any problem and in comparison, with the results at the time of release of the respective batch, no significant differences could be observed.

As for the temperature stress test selected media are comprising the most sensitive media PMM produces, the results can be considered as a good indication that plated media produced by PMM do not suffer from temperature stress up to 72 hours.

Neutralization of Disinfectants

The inactivation of residues of disinfectants is a hot topic during validation of contact plates. In EP 2.6.12 a table of “Interfering Substances” is listed together with the corresponding neutralizers (“Potential Neutralizing Method”). In this list in principle, all commonly used active ingredients of disinfectants are listed together with at least one neutralizing agent for such ingredients. Based on this enumeration, the commonly used TSA+LTHT media should neutralize all residues of disinfectants. However, whereas neutralizers on the one hand can neutralize harmful substances, they may have on the other hand themselves an inhibiting effect on the growth of some microorganisms (like thiosulfate in higher concentrations on Gram-positive bacteria). Therefore, the concentration of a neutralizer added to a medium need to be carefully established.

Experimental data show, that standard TSA plates with neutralizers are able to readily neutralize disinfectants containing ethanol, isopropanol, propanol, per-acetic acid, Na-hypochlorite or formaldehyde (e.g., Acticlens, Incidin, Buraton rapid, Biocide S, Klercide 70/30, Klercide Biocide C, Spitazid, SporGon, Spor-Klenz). Neutralization of these active substances is already accomplished by a basic TSA medium without neutralizers. Most probably the dilution is already sufficient enough, to abolish the activity of the disinfectant, so that the bacteriostatic or bactericide potency is completely lost.

Similarly, disinfectants containing Hydrogen-peroxide are (e.g., Klercide-CR Biocide C, Dec Spore 200 Plus or Spor-Klenz) can be inactivated by most of the media, especially when these are gamma-irradiated. This is due to the fact that during irradiation radicals are generated which have to be caught and inactivated to maintain the quality of the media. This is why gamma-irradiated media have to be supplemented by substances inactivating such radicals. Hydrogen-peroxide (or in general all peroxide containing disinfectants) also generates radicals as active substances, which are then inactivated by the radical inactivating supplements. Hydrogen-peroxide -containing disinfectants require in addition to the dilution an inactivation of the active substance. Such inactivation of hydrogen-peroxide will be accomplished by all gamma-irradiated plates provided by PMM.

Other disinfectants were only poorly inactivated by TSA+LTHT. When comparing these disinfectants (e.g., Gigasept AF, Klercide-CR Biocide X, Klercide-CR Biocide A, Klercide-CR Biocide B, Klercide-CR Biocide D, Amphospray, SteriClean Bio+ I and II and Melsept), it becomes obvious that they typically contain quaternary ammonium-compounds (QAC), sometimes in combination with other active substances like biguanides. The neutralization of these disinfectants requires a special neutralizer combination, like it is used in our TSA-U+ plates (Art.-No.: 101.0100) (see data below).

To compensate for the limited inactivation of residues it would have been obvious just to increase the concentration of the neutralizers added. However, it was shown that the even a strong increase in concentration of neutralizers was not sufficient to inactivate modern disinfectants. From about 2005, manufacturers of ready-to-use nutrient media have developed formula to inactivate QACs and biguanide-containing disinfectants. Although the plates developed at that time were able to inactivate QACs and biguanides partially, they are not really used by many companies, most probably due to some severe disadvantages, like short shelf-life, creation of precipitates, low recovery rates of some Gram-positive microorganisms or turbidity of the medium.

Inactivation of QAC disinfectants on TSA-LTHT

To confirm the ability to neutralize Quaternary Ammonium Compounds (QAC), we have performed a series of tests applying 50 µl of disinfectant per standard-contact plate (TSA+LTHT, art. 100.0100) followed by the determination of the recovery rates compared to plates not treated with disinfectant (see table “Inactivation of disinfectants on TSA-LTHT medium”).

Disinfectant	Concentration in mg/100ml		Test strain		
			S. aureus	*B. subtilis*	*S. epidermidis*
	QAC	Biguanide	µl disinfectant per TSA+LTHT contact plate		
			50	50	50
Gigasept AF (4%)	876		0	0	0
Hexaquart forte (2%)	558		0	0	0
Biocide A	500	200	0	0	0
Microbac forte (2%)	498		s. Microbac forte (0,5%)		
Terralin Protect (2%)	458		s. Terralin protect (0,5%)		
Gigasept AF (1,5%)	329		0	0	0
Biocide B	290		n.t.	n.t.	0
Microbac forte (2%)	249		n.t.	5	1
Sterillium classic pur	200		4	0	6
Lysoformin 3000 (2%)	192		s. Lysoformin 3000 (0,5%)		
Melsept SF (2%)	150		n.t.	0	0
Hexaquart forte (0,5%)	140		0	0	0
Microbac forte (0,5%)	125		n.t.	5	1
Korsolin FF (2%)	120		0	0	1
Hexanios G+R	117	12	n.t.	n.t.	0
Terralin Protect (0,5%)	115		n.t.	7	0
Amphospray 41 IP	109	96	0	0	0
Incidin Rapid (0,75%)	75		0	1	1
Lysoformin special (0,75%)	72	22	0	0	3
Lysoformin 3000 (0,5%)	48		n.t.	1	37
Melsept SF (0,5%)	38		n.t.	2	64
Korsolin FF (0,5%)	30		74	52	74
Incidin Rapid (0,25%)	25		81	59	69
Lysoformin special (0,25%)	24	7	33	1	0

The results reveal that only disinfectants containing very low concentrations of QACs were readily inactivated. Exceeds the concentration about 30mg of QAC per 100ml, sufficient neutralization was no more observed.

It can be concluded that a standard TSA+LTHT plate is inefficient for neutralization of such disinfectants. Expressed in other words this means that a standard TSA+LTHT medium is able to inactivate a maximum of about 15 µg QAC per plate. Based on the data observed it can be concluded, that surfaces disinfected with formula containing QACs need to be properly rinsed/washed to reduce the amounts of residues before surface samples with standard TSA+LTHT contact plates can be carried out.

Inactivation of disinfectant on TSA-U+ medium

Based on the results observed with the standard TSA+LTHT plates, there is a risk of obtaining false negative results when using disinfectants containing QAC and performing surface testing with a standard TSA-LTHT contact plate. PMM has developed a new medium to inactivate even very high concentrated active ingredients of disinfectants.

The newly developed TSA-U+ contact plate (art. 101.0100) was tested in a second series of tests, using the same disinfectants and the same test conditions. Results are shown in the table below.

The tests revealed that even the disinfectants with the highest concentration of QAC were inactivated without any problem, resulting in recovery rates well above 50%. Lower concentration disinfectants were not tested, if higher concentrated solutions of the same disinfectant were already shown to be inactivated (listed as “n.t.” in the table; e.g., Melsept, Lysoformin 3000).

TSA-U+ contact plates are able to inactivate at least 25 times higher concentrations of QACs and biguanides compared to the today commonly used contact plates with TSA+ LT or TSA+LTHT. In other words, TSA-U+ contact plate is able to inactivate more than 400µg QAC compared to a maximum of about 15 µg for a standard TSA-LTHT contact plate.

If disinfectants containing QAC are used and surfaces are sampled with standard TSA+U+ contact plates, reliable results can be obtained, even if residues of the disinfectants will be picked up during sampling. The disadvantages of former neutralizing plates have been completely eliminated (shelf-life of up to 9 months, the medium is clear, no precipitates, recovery rates of reference strains are well above 50%, even for *B. subtilis*).

Disinfectant	**Concentration in mg/100ml**		**Test Strain**						
			S. aureus	*B. subtilis*	*S. epidermidis*	*E. coli*	*Ps. aeruginosa*	*C. albicans*	*A. brasiliensis*
	QAC	**Biguanide**	µl disinfection per TSA-U+ contact plate						
			50	50	50	50	50	50	50
Gigasept AF (4%)	876		92	76	83	116	97	123	99
Hexaquart forte (2%)	558		86	106	79	92	101	97	108
Biocide A	500	200	111	109	126	105	95	112	89
Microbac forte (2%)	498		103	98	104	75	99	90	107
Terralin Protect (2%)	458		111	122	97	61	103	106	94
Biocide B	290		89	74	109	65	97	102	92
Microbac forte (2%)	249		103	98	104	75	99	90	107
Sterillium classic pur	200		102	106	102	n.t.	n.t.	n.t.	n.t.
Lysoformin 3000 (2%)	192		116	87	92	86	99	104	104
Melsept SF (2%)	150		110	60	131	76	93	106	133
Korsolin FF (2%)	120		64	94	82	n.t.	n.t.	n.t.	n.t.
Hexanios G+R	117	11,5	105	106	123	99	131	103	72
Amphospray 41 IP	109	96	87	93	89	85	127	127	119
Incidin Rapid (0,75%)	75		99	95	92	n.t.	n.t.	n.t.	n.t.
Lysoformin special (0,75%)	72	22	85	72	92	n.t.	n.t.	n.t.	n.t.
Lysoformin 3000 (0,5%)	48		109	77	107	n.t.	n.t.	n.t.	n.t.

Plated media containing Penase and/or β-Lactamase-II

Whereas standard TSA media plates are pretty easy to produce, TSA media containing enzymes are much more difficult to produce. For use in the Pharmaceutical industry mainly plates containing either Penase, β-Lactamase II or a mixture of both are of interest. In the following a summary of the different topics to be considered when manufacturing gamma-irradiated plates with these enzymes are listed.

First at all the different enzymes need to looked at in a little bit more detail:

Penase, also named β-Lactamase I – active against β-lactam antibiotics of the class of Penicillins. Penase does hardly show any activity against cephalosporins, if at all:

Although Penase can be purchased by different suppliers, the enzyme supplied by these suppliers is always the same. Therefore, the activity of the enzyme is solely depending on the amount of Penase added to the medium and the amount of activity lost during the production process and the irradiation.

β-Lactamase-II, also named Cephase, Lactamator, Carbamator, Carbamator plus ……. – active against β-lactam antibiotics of the class of Penicillins as well as cephalosporins.
Whereas β-Lactamase-II was originally extracted from *B. cereus*, newer enzymes have been detected due to the more and more occurring resistances against cephalosporins in medical treatments. Such enzymes responsible for these resistances have been identified, isolated, cloned and finally over-expressed in production strains like *E. coli*. The different enzymes all show activity against cephalosporins, however, the substrate spectrum and the activity against a specific cephalosporin.
Furthermore β-Lactamase showing different sensitivities towards heat treatment or to irradiation. Therefore, the enzyme activity of a β-lactamase II may differ depending on the enzyme used, as well as on the media preparation and the irradiation. The more sensitive an enzyme is the more activity is lost during the manufacturing and irradiation process. Consequently, the media lots produced may show differences in enzyme activity between the beginning and the end of a lot and furthermore depending on the irradiation doses applied for the irradiation of the plated media. Most likely, the majority of enzyme activity of most β-lactamases II used in β-Lactamase II plates is destroyed during the preparation of the media and only the minor part of the activity is available for the inactivation of cephalosporins getting into contact with the plates.

For the reasons mentioned above, it is difficult to compare plates containing different enzymes. However, PMM has decided to use enzymes, which are almost stable during the manufacturing and irradiation process. β-Lactamase 2G is a newly developed broad spectrum β-lactamase which is able to inactivate penicillins, the vast majority of the 1st, 2nd, 3rd, 4th and 5th generation cephalosporins as well as carbapenems. Furthermore, Lactamase 2G is the showing improved stability, thus guaranteeing the highest degree in reliability and stability compared β-lactamases available so far. Lactamase 2G plates are especially designed to be used in environments where higher concentrations of cephalosporins or carbapenems may interfere with the microbial monitoring.

β-lactamase-II plates testing:
Comparing β-lactamase-II plates: Cephalosporins are active against a wide variety of different microorganisms. However, in case you would like to perform a comparison of different β-Lactamase plates, please use the *E. coli* strain listed in the EP/USP. This strain is highly sensitive towards the action of cephalosporins; therefore, it is allowing very sensitive detection of different β-lactamase-II activities in different types of β-lactamase plates.

Which neutralizers to be used with β-lactamase-II plates: Whereas most plates used for environmental monitoring do contain Lecithin, Tween 80 (Polysorbate) Histidine and Thiosulfate, β-lactamase-II plates **must not** contain Histidine and Thiosulfate. Histidine/Thiosulfate has been shown to inhibit the activity of β-lactamases-II towards some cephalosporins. PMM β-lactamase-II plates therefore contain either Lecithin, Tween 80 and Glycine (TSA+LTH + β-Lac 2G) or just Lecithin and Tween 80 (TSA+LT + β-Lac-I/II).

General characteristics of CSG and CS products

PMM plated media are made for applications in industry, laboratories and research institutions. The products are named differently according to their main application:

CSG products are made mainly for the application in critical environments, e.g., in isolators, clean rooms classes A and B as well as in RABS. CSG products are triple bagged and gamma-irradiated at 9 to 20 kGy.

CS products are single bagged and not irradiated – therefore CS plates should be used only in laboratories and clean rooms class C and D, but must not be used in clean rooms class A and B, isolators and RABS. Besides some media for environmental monitoring media for the detection of specified microorganisms fall into this group.

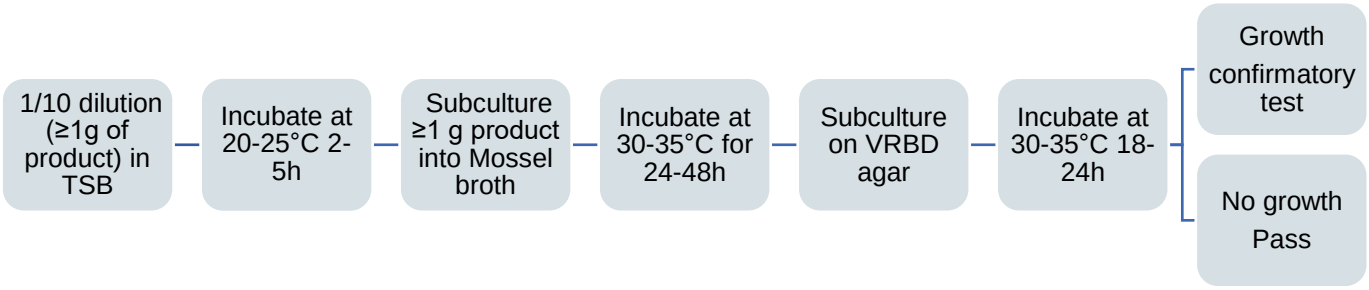
	CSG – Click & Safe - gamma	CS – Click & Safe
Application	• Environmental Monitoring in Cleanrooms, RABS and Isolators: - Surface - Personnel - Air	• Hygiene Monitoring in less- critical areas as well as product testing: - Surface - Personnel - Air - Water - Product • Detection of specified aerobic micro-organisms
Type	• TSA (only 90mm plates) • TSA with neutralizer • SDA with neutralizer • TSA with β-Lactamase Plus • TSA with Penase	• TSA • TSA with neutralizer • SDA • R2A • Selective media
Petri dishes/ package	• Lockable Petri dishes • 90mm (30ml-filling) • 55 mm (18ml-filling) • Triple bagged, 10 plates in H_2O_2-impermeable bag with desiccant • 90mm plates: 60 units per box • Contact plates: 100 units per box	• Lockable petri dishes 90 mm (24/30ml-filling) 55 mm (18ml-filling) • Single bagged 10 plates with desiccant • 90mm plates: 60 units per box • Contact plates: 120 units per box
Irradiation	Gamma-irradiated 9-20KGy	Not irradiated
Storage	15°C-25°C	
Shelf-life	Refer to Product list	
Benefit	✓ Safe transport and handling with lockable Petri dishes ✓ Reduced condensation ✓ Large batches = minus incoming goods controls ✓ Long incubations ✓ Unique identification number on each plate ✓ Barcode on each plate ✓ temperature-insulated packaging	

Please be aware that PMM products do not have to be registered according to the **IVD** guideline, as the products are not intended to be used in clinical or medical diagnostic applications.

Selective media testing

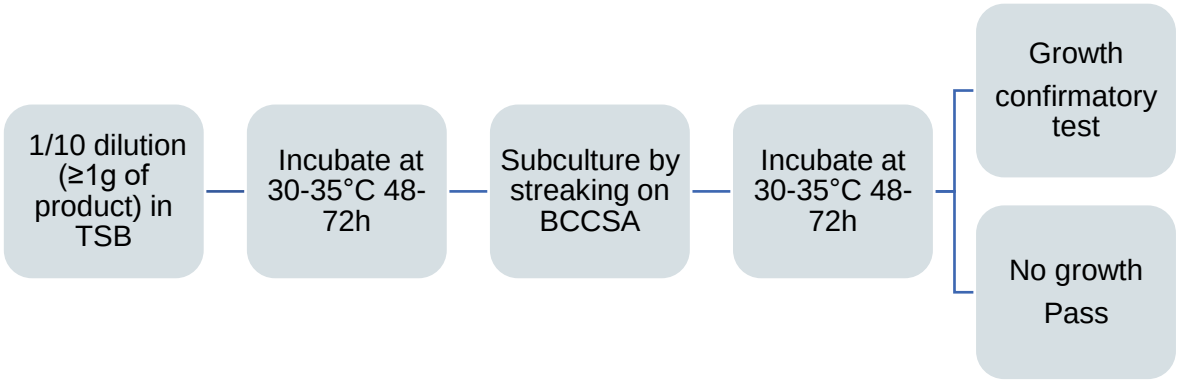
PMM provide selective culture media in compliance with pharmaceutical applications: EP2.6.13/USP <62> and USP <60> for the detection of specified microorganisms.

Absence of Bile-tolerant Gram-negative bacteria



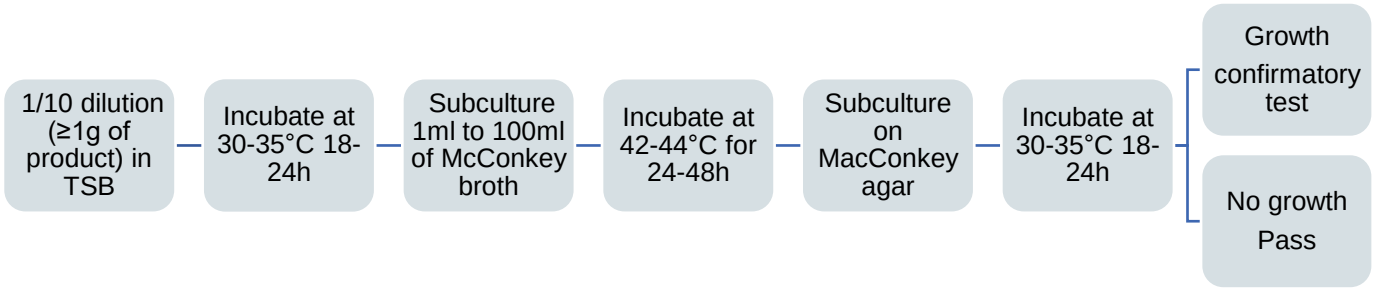
Article n°	Culture media name	Format
465.0060	VRBD Agar 90mm	Box of 60 plates, single wrapped
500.B100	TSB 100ml	12 screw cap bottles per rack
503.B200	TSB 200ml	12 screw cap bottles per rack
505.D000	TSB 1000ml	6 screw cap bottles per rack

Absence of Burkholderia cepacia complex



Article n°	Culture media name	Format
451.0060	*Burkholderia cepacia* Complex Selective Agar 90mm	Box of 60 plates, single wrapped
500.B100	TSB 100ml	12 screw cap bottles per rack
503.B200	TSB 200ml	12 screw cap bottles per rack
505.D000	TSB 1000ml	6 screw cap bottles per rack

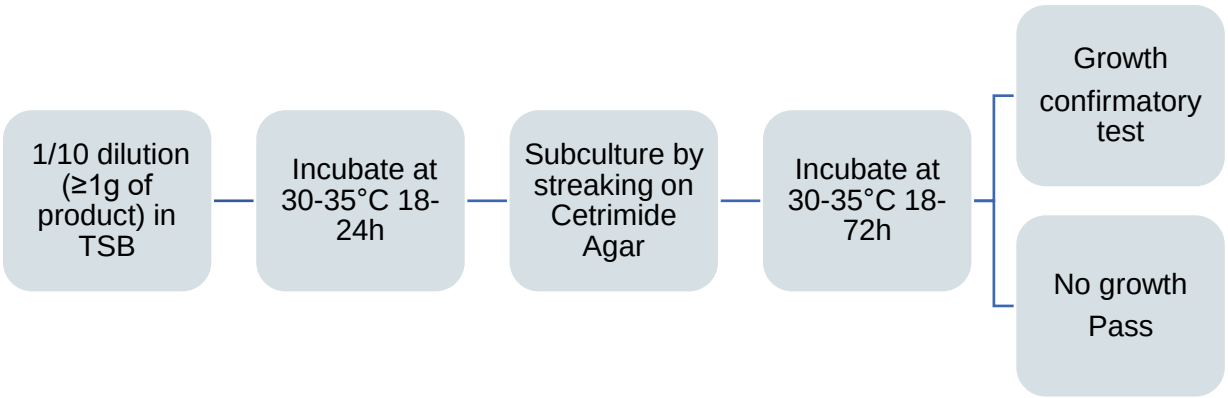
Absence of Escherichia coli



Culture media required:

Article n°	Culture media name	Format
460.0060	MacConkey Agar 90mm	Box of 60 plates, single wrapped
581.B100	MacConkey Broth	12 screw cap bottles per rack
500.B100	TSB 100ml	12 screw cap bottles per rack
503.B200	TSB 200ml	12 screw cap bottles per rack
505.D000	TSB 1000ml	6 screw cap bottles per rack

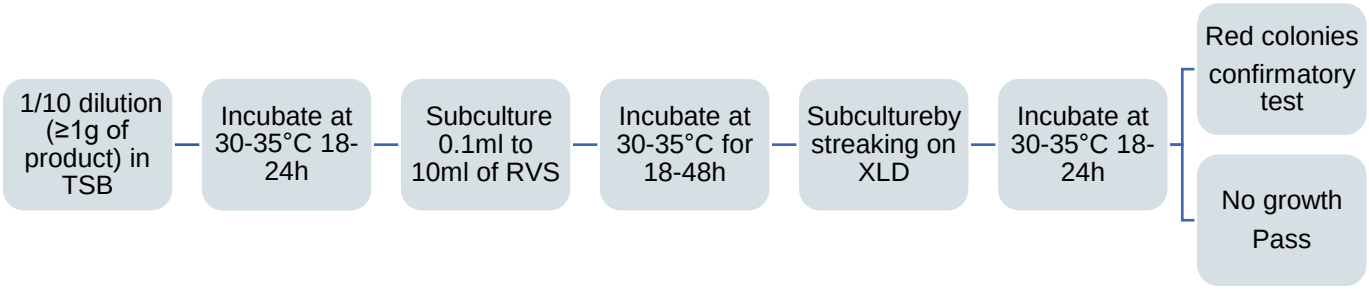
Absence of Pseudomonas aeruginosa



Culture media required:

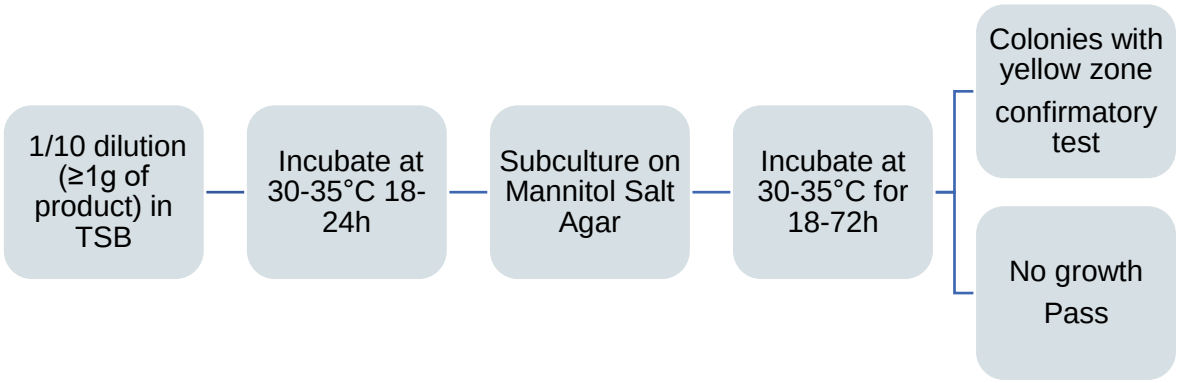
Article n°	Culture media name	Format
450.0060	Cetrimide Agar 90mm	Box of 60 plates, single wrapped
500.B100	TSB 100ml	12 screw cap bottles per rack
503.B200	TSB 200ml	12 screw cap bottles per rack
505.D000	TSB 1000ml	6 screw cap bottles per rack

Absence of Salmonella spp.



Article n°	Culture media name	Format
470.0060	XLD Agar 90mm	Box of 60 plates, single wrapped
500.B100	TSB 100ml	12 screw cap bottles per rack
503.B200	TSB 200ml	12 screw cap bottles per rack
505.D000	TSB 1000ml	6 screw cap bottles per rack

Absence of Staphylococcus aureus



Article n°	Culture media name	Format
455.0060	Mannitol Salt Agar 90mm	Box of 60 plates, single wrapped
500.B100	TSB 100ml	12 screw cap bottles per rack
503.B200	TSB 200ml	12 screw cap bottles per rack
505.D000	TSB 1000ml	6 screw cap bottles per rack

Bottled Media

Bottled media are available either as standard products or alternatively as customized products. For the production of bottles, we do have different options – please find below a list of the available bottles as well as available closures

Standard 220 ml bottle (glass quality type II)

Nominal volume	Weight (g)	Weight lid (g)	Filling vol. (max)	Height (mm)	Diameter (mm)	Opening (mm)	Closure
200ml	190	16,5	250	115,6 1,1	69 ± 1,0	31,5	GL40

Laboratory Glass Bottles (Borosilicate type glass, type I)

Nominal volume	Weight (g)	Weight lid (g)	Filling vol. (max)	Height (mm)	Diameter (mm)	Opening (mm)	Closure
250ml	228	26	290	138 ± 1,5	70 ± 1	29,8 ± 0,5	GL 45 x 4
500ml	342	26	600	176 ± 1,5	86 ± 1,5	29,8 ± 0,5	GL 45 x 4
1000ml	569	26	1100	225 ± 1,5	101 ± 1,5	29,8 ± 0,5	GL 45 x 4

Infusion Bottles (glass quality type II)

Nominal volume	Weight (g)	Weight lid (g)	Filling vol. (max)	Height (mm)	Diameter (mm)	Opening (mm)	Closure
100ml	92	11	128	104 ± 0,8	49 ± 0,8	20	ISO 3536-1-A
250ml	170	11	308	136 ± 1,2	66 ± 1,2	20	ISO 3536-1-A
500ml	290	11	588	177 ± 1,4	78 ± 1,4	20	ISO 3536-1-A
1000ml	385	11	1120	225 ± 1,5	95 ± 1,3	20	ISO 3536-1-A

Bottled media produced at PMM are terminally sterilized by autoclaving. Therefore, bottles do not need to be produced in clean room zones classified according to classes A, B, C or D. However, bottles are produced in a specific hygiene zone; additionally, the filling area is protected by a laminar flow zone.

Due to the special autoclaving process in a sprinkling autoclave, bottles produced at PMM are closed before sterilization, thus avoiding any unnecessary handling of the bottles after autoclaving.

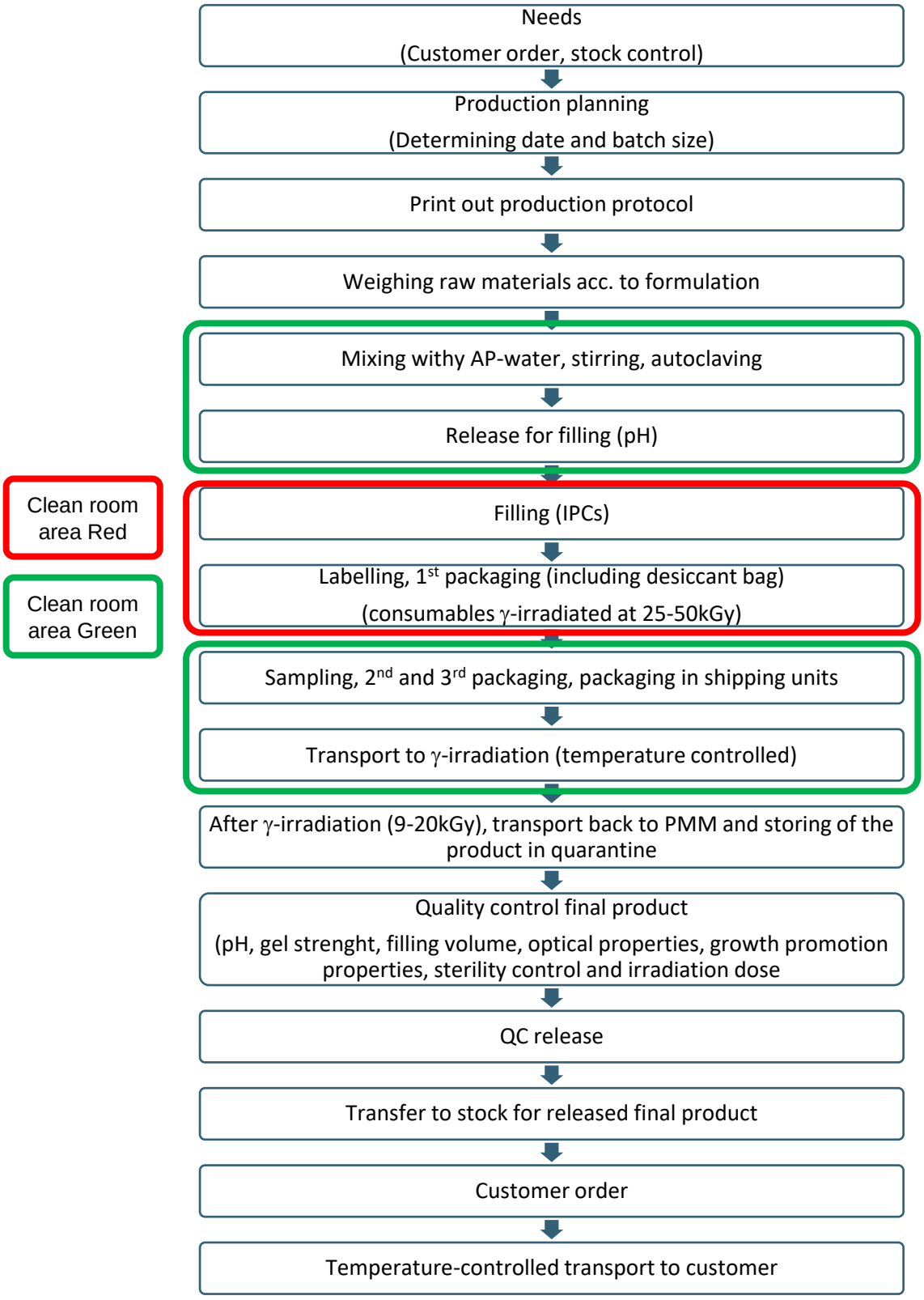
Please find below a Process Flow Chart for bottles produced at PMM:

Bottles produced at PMM show the following features:

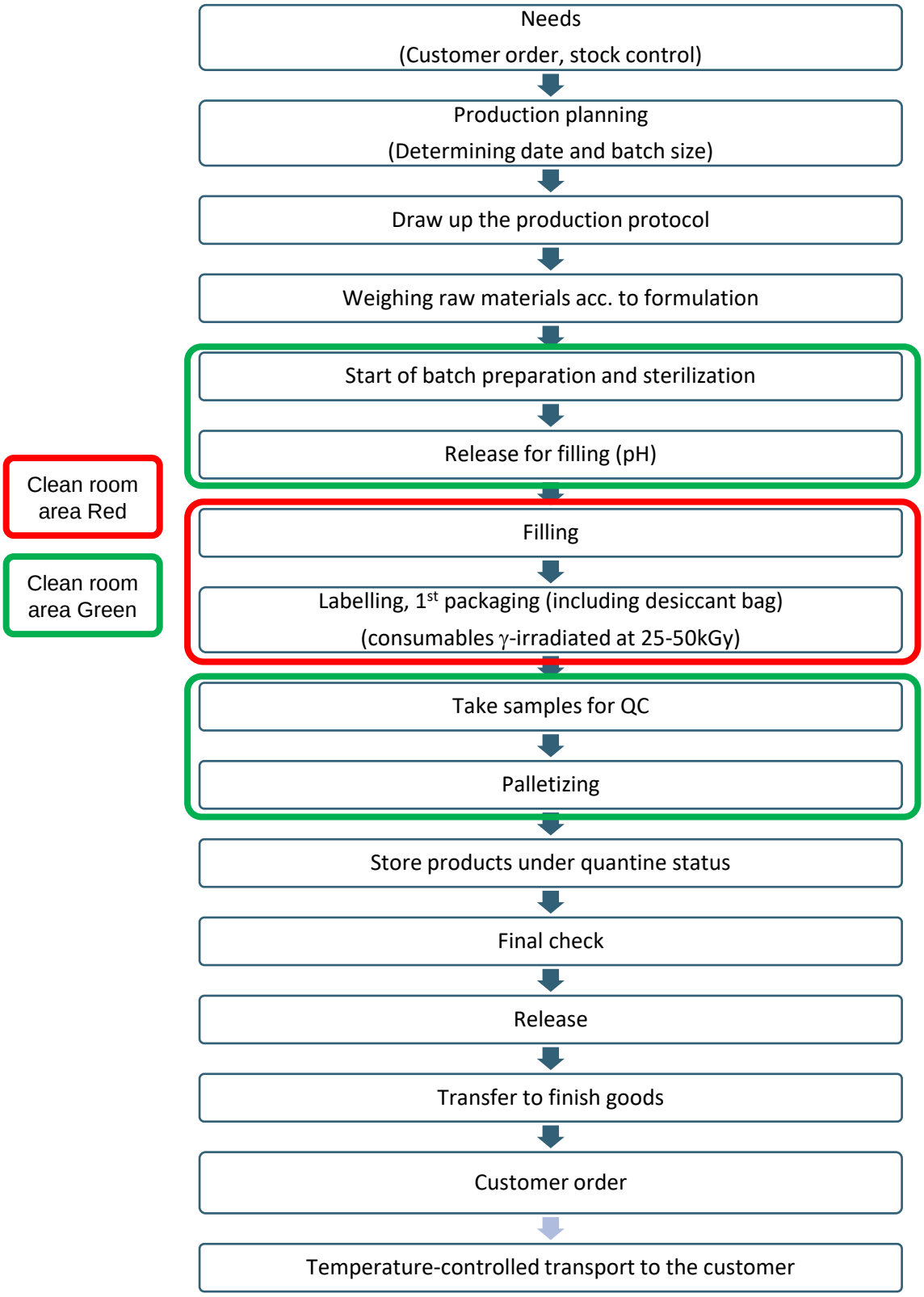
- Labels with autoclaving indicator
- Labels with data matrix bar code ECC 200 (s. chapter labelling information)
- Each bottle contains a unique identification number
- Bottles with color-coded closures
- All bottles are incubated after autoclaving for at least 48 hours
- No handling of bottles with unprotected hands after autoclaving
- Packaging of bottles in tray with shrink foil after autoclaving to reduce the bioburden on the bottles
- Delivery of bottles either in trays with shrink foil or alternatively in reusable boxes (deposit system)

Manufacturing flow charts

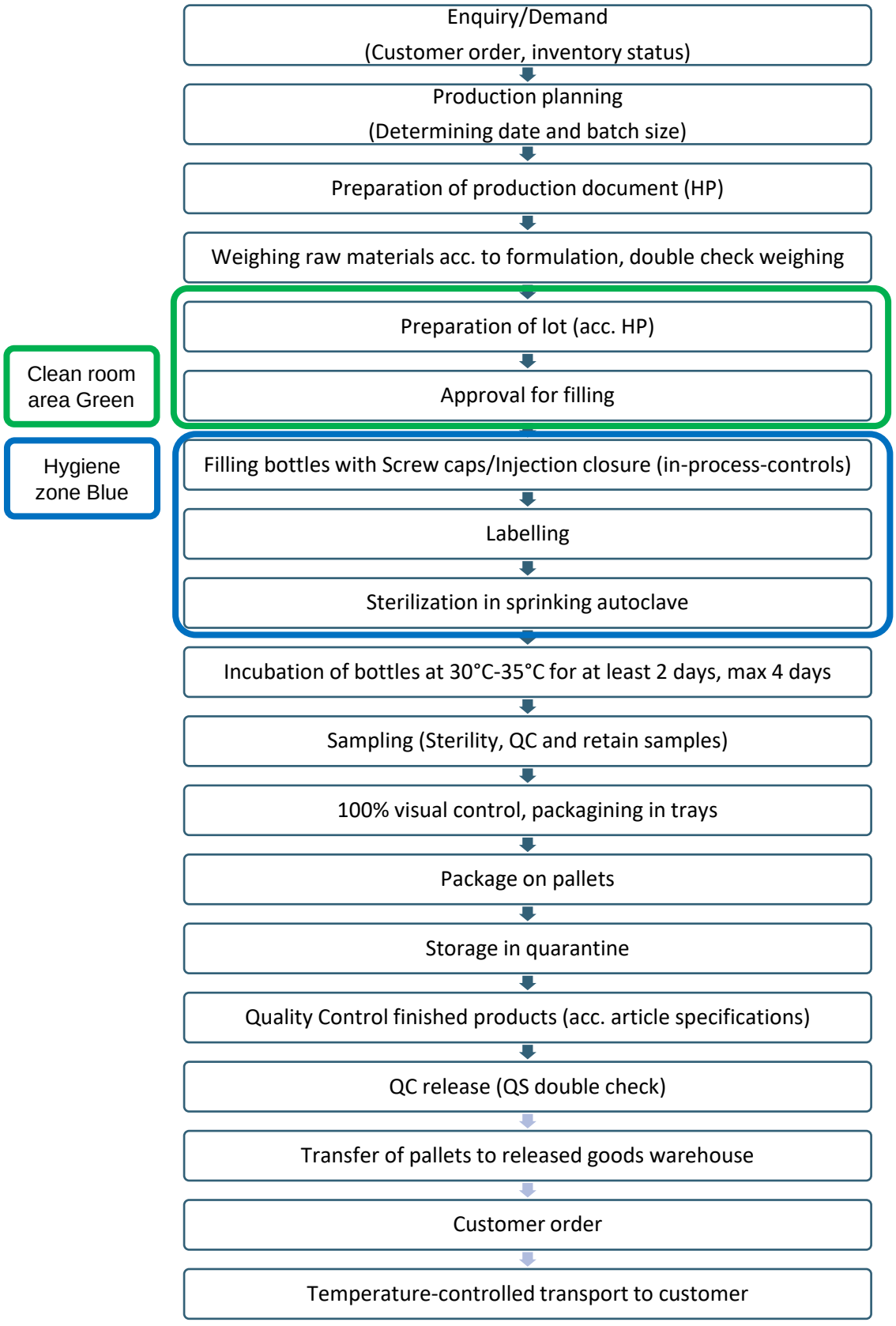
Process flow chart – Click & Safe Gamma Irradiated plates



Process flow chart – CS Click & Safe plates



Process Flow Chart – Bottled Media



Customized Product Request

	Contact details
Company	
Address	
Contact person	
Job title	
Email	
Phone	

	Culture medium
Expected delivery	Month/Year: _____/_____
Expected shelf life	_____
Storage temperature	_____
Composition	

Ingredient	Quantity/l

	Culture medium (Plated media)
Petri dish size	☐ 55mm (Contact) ☐ 90mm (Settle) ☐ Other: _____
Filling volume	☐ 18ml ☐ 30ml ☐ Other: _____
Gamma-irradiated Triple wrapped	☐ Yes ☐ No
Plates per batch	_____
Plates per year	_____

	Culture medium (Bottled media)
Bottle size	☐ 100ml ☐ 200ml ☐ 500ml ☐ 1000ml ☐ Other: _____
Type of bottle	☐ Screw cap ☐ Infusion ☐ Other: _____
Filling volume	☐ Yes ☐ No
Screw cap colour	☐ Red ☐ Green ☐ Blue
Flip cap colour	☐ Red ☐ Green ☐ Blue ☐ White ☐ Yellow
Bottles per batch	_____
Bottles per year	_____

	Media Fill
Format description	

	Quality control
Composition	
Additional tests	

Genus and species	Collection number

_____

Date / Signature