sartorius

Validation Guide
for Nutrient Pad Sets

Table of Contents

1. Introduction

Pharmaceutical products, such as injectable and infusion solutions or those that come in contact with open wounds, must conform to exactly defined quality standards. The desired quality of the final product can only be obtained when the entire production process is adequately safeguarded against contamination, particularly at critical points where this product is exposed to microbial contamination. An aseptic manufacturing process must be validated, taking all aspects of the product and the production process into account.

Validation is indispensable for guaranteeing the safety of pharmaceuticals. It is therefore a logical supplement and significant part of the cGMP regulations, which have been in force for quite some time. Guidelines for validation have been jointly established by the Committee for Laboratories and Official Drug Product Inspection Services and the Department of Industrial Pharmacists of the Féderation Internationale Pharmaceutique (F.I.P.), which is the European counterpart of the U.S. Food and Drug Administration.

These guidelines define validation as follows:

"Validation, as used in these guidelines, comprises the systematic testing of essential production steps and equipment in the R & D and production departments, including testing and inspection of pharmaceutical products, with the goal of ensuring that the finished products can be manufactured reliably and reproducibly and in the desired quality in keeping with the established production and quality control procedures."

During product validation, Sartorius also follows the "EEC guide to good manufacturing practice for medicinal products" published by the Commission of the European Communities.

We have compiled this Validation Guide so that users of Sartorius Nutrient Pad Sets can plan, implement and document their own validation procedures.

Growing requirements are placed on the microbiological and hygienic quality of products, whether they are foods and beverages, pharmaceuticals and cosmetics or articles from many areas of daily use.

These requirements result from consumer demands, such as the demand for a longer shelf life, and from the logistics of transportation routes that are becoming more and more extensive because products and raw materials are increasingly being shipped all over the world. In addition, the consumers' increased awareness of hygiene as well as legal standards of hygiene for many products are following this trend toward stricter microbiological and hygienic standards. Food processors are requiring that their suppliers meet in-house standards on maximum or limit colony-forming units and that ingredients not contain any product-spoilage microbes. As a result, microbiological testing is growing in importance for assessing the quality of both raw materials and finished products.

For this reason, there is a need beyond the pharmaceutical area for simple, reliable and quick testing methods that deliver reproducible results, but that do not need extensive preparation and that require as few personnel and as low an amount of laboratory equipment as possible. Sartorius Nutrient Pad Sets optimally meet these requirements.

1.1 cGMP Quality from Sartorius
The consistently high quality of Sartorius Nutrient Pad Sets is assured by careful selection of raw materials, well-planned and validated production technologies, and extraordinarily effective quality assurance measures. As a result, Sartorius Nutrient Pad Sets provide high batch-to-batch reproducibility. The quality control test procedures used at Sartorius are based on external and in-house standard methods that have resulted from Sartorius' many years of experience with these products.

1.2 Quality Assurance
Within the scope of quality assurance at Sartorius, all materials for Nutrient Pad Sets have been carefully selected in conformance with stringent in-house standards and supplier specifications imposed by the Sartorius Purchasing Department.

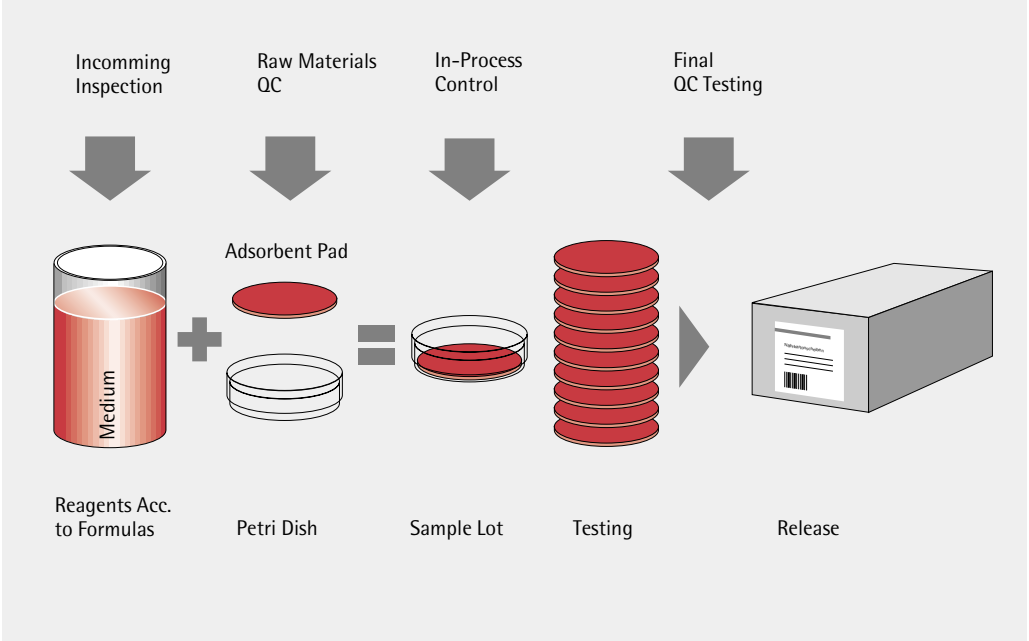
Quality is documented starting with incoming inspection of the raw, auxiliary and packaging materials for Nutrient Pad Sets.

Adherence to GMP requirements (clean-room conditions, gowning and employee hygiene and training) ensures optimal quality control in standard operating procedures for production, which are monitored by documented in-process controls performed at regular intervals (airborne microbe sampling, monitoring of surface contamination, and similar tests).

Moreover, Sartorius Nutrient Pad Sets must pass rigorous final quality control testing. These individual QC tests are conducted using a defined random sample of Nutrient Pad Sets.

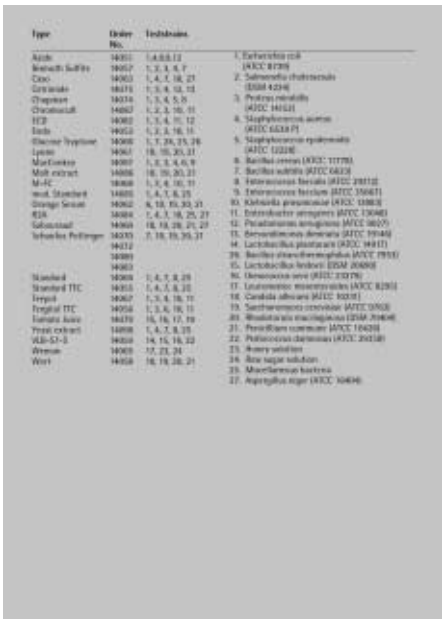
1.3 Prevention of Contamination
The production conditions are designed to prevent contamination as far as possible during manufacture of Sartorius Nutrient Pad Sets. Procedures for lowering the bioburden have been implemented at several points within the manufacturing process.

Manufacture of Nutrient Pad Sets



1.4 Completely Traceable Documentation

The type and lot number as well as the sterilization and expiration dates are printed on the packaging labels to identify Sartorius Nutrient Pad Sets. Each lot number enables Sartorius to trace and recall all data on production steps, tests and materials used. Samples of every lot are placed on file to ensure traceability at least for the entire length of their indicated expiration date. All parameters concerning the initial materials, manufacturing conditions and tests are summarized in an accompanying report that is the basis on which the quality certificate for each lot is issued. This certificate is included in every box of Nutrient Pad Sets.



1.5 DIN ISO Certificate

The quality management system of Sartorius AG meets the requirements of DIN EN ISO 9001, which is the national equivalent of the International Standard ISO 9001, and has been certified by DQS, the German Association for the Certification of Quality Systems.

1.6 GMP Certificate

The District Administration of Braunschweig, Germany, has certified that the manufacture and quality control of Nutrient Pad Sets at Sartorius AG comply with GMP requirements.

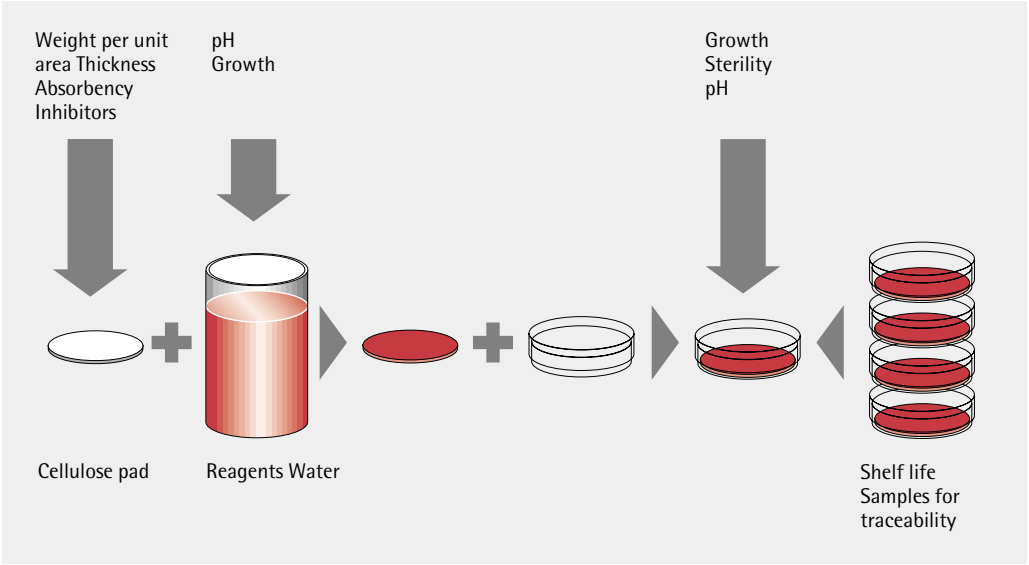
2. Technical Specifications

2.1 What Are Nutrient Pad Sets?
Nutrient Pad Sets (German abbreviation NKS) are ready-to-use, dehydrated, solid culture media. They consist of biologically inert cellulose pads that have been impregnated with liquid nutrient media of the formula corresponding to their type, and sterilized in this condition.

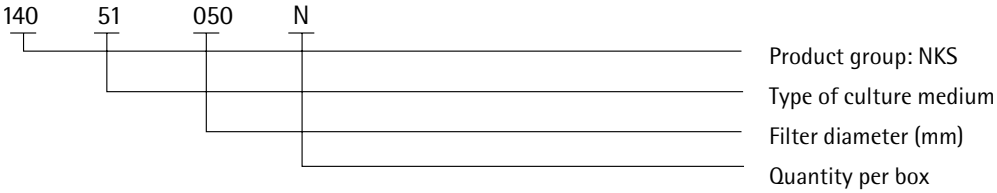
Nutrient pads are individually pre-plated in sterile petri dishes. For use, the nutrient pads only need to be moistened with 3.5 ml of sterile, demineralized water. Because nutrient pads are always used in conjunction with membrane filters, each type of nutrient pad comes with individually packaged and presterilized membrane filters that are ideally suitable for the specific application.

Therefore, each package of Nutrient Pad Sets contains all expendables that the user requires for microbiological testing: culture media, filters and culture plates (petri dishes). These Sets rule out problems with sufficient cooling capacity because they can always be stored at room temperature. In addition, they eliminate the need for a large amount of laboratory equipment, such as an autoclave, water bath, pH meter, drying oven, etc.

Tests and Controls during Manufacture of Nutrient Pad Sets



Order Number Code:



All types of Nutrient Pad Sets are available with 47- or 50-mm diameter filters.
The Sets are available in boxed quantities of 100 (ID letter code N).
The Nutrient Pad Set types Endo, Standard TTC and Wort are also available in a special version without petri dishes; these versions have the following order numbers:

Endo 14003, Standard TTC 14005, Wort 14008.
The ID letter codes for the filter diameter and the quantity per box, "K," must be added to these basic order numbers.

2.2 Types, Order Numbers, Application Information, Evaluation

Type	Order No.	For Detection of	Application	Incubation Conditions	pH	Results
Azide	14051	Enterococci	Water, beverages, foods, raw materials	up to 48 hrs. at 37°C	7.2±0.2	brownish-red colonies, diameter up to 1 mm
Bismuth -Sulfite	14057	Salmonella typhi and other types of salmonellae	Water, waste water and other samples	up to 48 hrs. at 37°C	7.6±0.2	Light-colored area surrounding a black center; zone plain black or black with a metallic sheen
Caso	14063	Colony count (CFU)	Pharmaceuticals and cosmetics, water	24–72 hrs. at 30–37°C	7.3±0.2	Predominantly bacteria with colonies of different shapes and colors
Cetrimide	14075	Pseudomonas aeruginosa	Water, beverages, pharmaceuticals, cosmetics, raw materials, food	48 hrs. at 37°C	7.2±0.2	Ps. aeruginosa usually forms blue colonies with blue zones
Chapman	14074	Staphylococcus aureus and other staphylococci	Pharmaceuticals and cosmetics, raw materials, foods	48 hrs. at 37°C	7.4±0.2	Staph. aureus develops golden-yellow colonies; other staph., whitish colonies
Chromo-cult	14087	E. coli and coliforms	Water, foods, beverages and other products	24 hrs. at 35–37°C	7.4±0.2	E. coli develop dark blue to violet colonies, coliforms salmon to red colonies
ECD	14082	Escherichia coli	All products and raw materials	18–24 hrs. at 37°C	7.0±0.2	E. coli colonies are fluorescent under UV light, 360 nm
Endo	14053 14003	E. coli and coliforms	Water, waste water, foods, beverages and other products and raw materials,	24 hours at 37°C	7.4±0.2	Red colonies; E. coli usually has a metallic sheen; by contrast, coliforms lack this sheen ingredients
Glucose-Tryptone	14066	Thermophilic spore formers	Sugar, sugar products and canned foods	2 days at 55°C	6.8±0.2	Bac.stearothermophilus and "flat-sour" colonies are yellowish-green with yellow zones; 2–5 mm ∅
Lysine	14061	Wild yeasts	Beer	2–5 days at 25–28°C	5.0±0.2	White to cream-colored colonies; only these yeasts metabolize lysine.
Mac Conkey	14097	Enterobacteria	Pharmaceuticals, water, beverage, food	18–24 hrs. at 37°C	7.1±0.2	Gram-negative enterobacteria form colonies of various colors
Malt extract	14086	Yeasts and molds	Soft drinks and other beverages	2–3 days approx. 25–30°C	3.5±0.2	Yeasts smoothy, shiny mostly white; cotton – wool – like molds.
MFC	14068	E. coli and coliforms	Water, waste water, food beverages and other products	20±4 hrs. at 37°C (44°C in a water bath)	7.4±0.2	Lactose-positive microbes develop blue colonies with blue zones
Orange Serum	14062	Acid-tolerant microbes	Raw materials, foods, beverages and other products	2–5 days at 25–28°C; aerobic or anaerobic	5.5±0.2	Acid-tolerant bacteria, yeasts and molds
R2A	14084	Colony Count	Water, waste water, water for pharmaceutical purpose	48–72 h | 35°C 5–7 days | 22°C	7.2±0.2	Colonies of different shapes and color Predominantly bacteria.
Sabou-raud	14069	Yeasts and molds	Pharmaceuticals and cosmetics, raw materials, water	2–5 days at 25–30°C	5.4±0.2	Yeasts develop smooth, shiny, and white colonies; molds form fluffy, cotton-like, white colonies
Schaufus-Pottinger	14070 14072	Yeasts and molds	Soft drinks and sugar	2–3 days at 28–30°C	4.3±0.2	Sugar fermenters develop whitish-yellow colonies; other colonies are bluish-green
Standard	14064	Colony count	Raw materials, water, waste water, beverages, foods, and other products	at least 48 hrs. at approx. 30°C	7.2±0.2	Predominantly bacteria colonies of various sizes, shapes and color.
Standard TTC	14055 14005	Colony count of aerobic mesophilic microorganisms	Raw materials, water, waste water, foods, beverages and other products	48 hours at 30°C	7.2±0.2	Predominantly bacteria colonies that are usually colorless and have various sizes and shapes
Teepol	14067	E. coli and coliforms	Water, waste water, foods, beverages and other products	18–24 hrs. at 37°C	7.2±0.2	Lactose-positive organisms form yellow colonies with yellow zones
Tergitol TTC	14056	E. coli and coliforms	Water, waste water, foods, beverages, pharmaceuticals and cosmetics	20 ±4 hrs. at 37°C	8.0±0.2	E. coli, yellow with yellow zones; coliforms, red colonies, some of which may have yellow zones
Tomato Juice	14079	Leuconostoc oenos and other lactic-acid spoilage bacteria	Wine and fruit juice	4–6 days at 25–30°C anaerobic	4.4±0.2	Lactic-acid bacteria form small, whitish colonies; Leuconostoc colonies are <1mm ∅
VLB-S7-S	14059	Beer-spoilage bacteria, particularly lactobacilli and pediococci	Beer and other products	5–7 days at 25–28°C anaerobic to microaerophilic	5.5±0.2	Beer spoiling organisms form greenish colonies Formation of acid changes color to yellow
Weman	14065	Mesophilic, slime-forming colonies (Leuconostoc mes.)	Sugar, concentrates and soft drinks	2–3 days at 28–30°C	5.5±0.2	Leuconostoc mesenteroides forms transparent, colorless colonies, some with diameters greater than 5 mm.
Wort	14058 14008	Yeasts and molds	Raw materials, foods, beverages and other products	2–3 days at approx. 25–30°C	4.4±0.2	Yeasts smoothy, shiny, white or colored; cotton-wool-like molds, first white then in different colors.
Yeast extract	14090	Colony count (CFU)	Water, waste water	44 ±4 hrs. 36 ±2°C 68 ±4 hrs. 22 ±2°C	7.2±0.2	Bacteria, yeasts molds can grow, most colorless.

2.3 Advantages of Nutrient Pad Sets

Economical.
No preparation of culture media; no cleaning or sterilization of culture glassware and equipment.

Simple to use.
No additional equipment is necessary. Water can be sterilized using a dosing syringe and an attached sterilizing syringe filter holder.

Consistent quality.
Stringent quality control assures consistent quality and guarantees reproducible results at the user's facilities. Nutrient Pad Sets are manufactured under GMP conditions.

Trouble-free storage.
Storage of the Sets in a dark, dry place at room temperature (approx. 20°C) ensures that they can be used right up to the expiration date printed on the label.

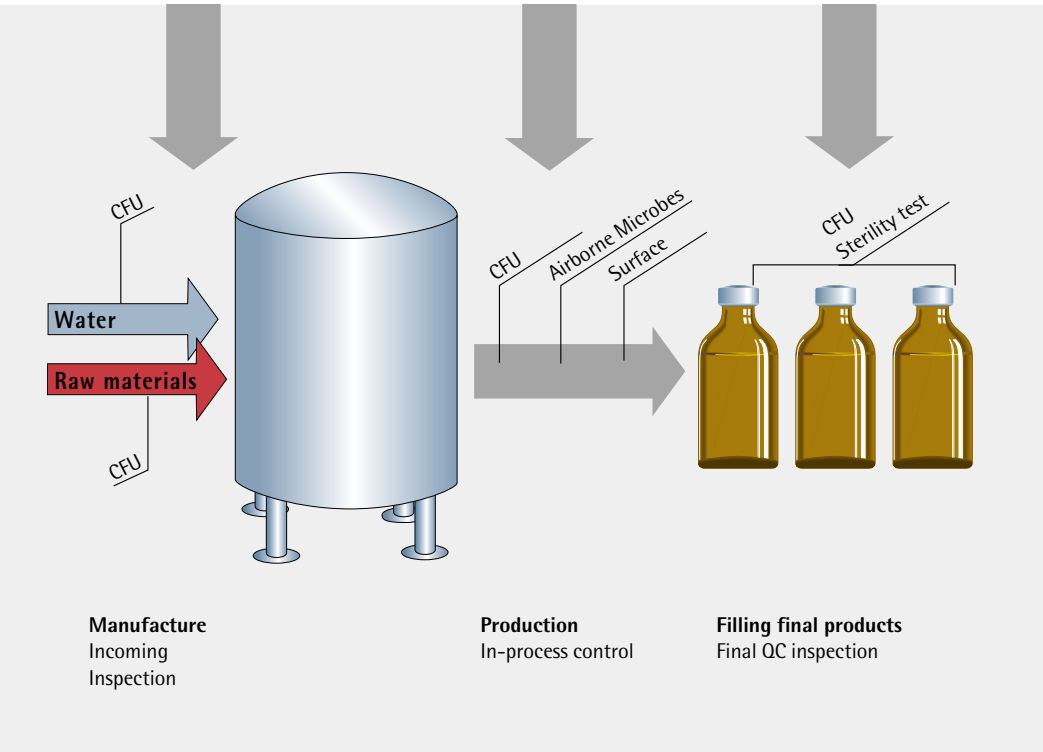
Versatile.
The user can modify the selectivity or specificity of the nutrient pads by putting additives in the solution used to moisten the pads; e.g., antibiotics for inhibiting unwanted accompanying flora, sugar for specific detection of osmophilic microbes, or acids or bases to change the pH, etc.

2.4 Applications

The major area of application for Sartorius Nutrient Pad Sets is microbiological testing of relatively large sample volumes that have a low number of microbes, with membrane filtration being an essential step. Of course, these Nutrient Pad Sets are also suitable for testing smaller volumes by placing a sample in a few ml of sterile water, for instance, directly in the filter holder. It is also possible to streak a specimen on the Nutrient Pads Sets, if the filter was previously placed on the moistened pad. In addition, Nutrient Pad Sets are suitable for the direct contact test method. In this case, a dry filter (if the surface to check is moist) or a prewetted filter (if the surface to check is dry) is pressed onto a surface to be examined, then placed on a moistened Nutrient Pad.

During the manufacture of products, the CFU counts must be determined at many levels in order to maintain control over the microbiological and hygienic quality of these products.

Applications in Process-Scale Manufacture



Incoming Inspection.
In many cases, incoming raw materials have a high bioburden, particularly if these materials are products of animal or plant origin. For some raw materials, there are limits for the total number of colony-forming units (CFU count), and/or the total absence of special problem microbes in defined volumes may be prescribed.

Water.
Water as an ingredient must comply with drinking water regulations in various countries. These regulations specify that the quality of treated water may not in any case be lower than that of the other raw materials. However, after water has been treated, it must have even fewer CFU counts in order to be used as an ingredient. This makes it easier to determine the effective bioburden by using membrane filtration.

Mixing tank.
If water and raw materials used as the ingredients are not sterile in the mixing tank, it can always be expected that the microbes will propagate and be carried over from the mixing tank to all subsequent processing steps. For this reason, it is important to determine the CFU count at this stage as well as in the following steps. The CFU count is a reference value for the later sterilization procedure.

The greater the bioburden of the material to be sterilized, the higher the amount of work will be needed for later sterilization.

Production systems.
CFU counts are also required to monitor the hygiene of equipment, systems, installations, personnel, facilities, etc., to ensure that all manufacturing operations comply with GMP. In most cases, Nutrient Pad Sets are suitable for this test application because the sterile filter included can be used as a "contact plate" that is subsequently incubated on the nutrient pad.

Final Product.
Sterility testing is frequently performed as quality control inspection of the final product in the manufacture of pharmaceuticals. For non-sterile products, such as oral preparations and skin care products, CFU limits are prescribed. Nutrient Pad Sets can be used to determine the CFU or colony count of these products.

If the sample to be tested is an easy-to-filter liquid, preparing it for microbial detection is simple: any particular volume of this sample is filtered first, then the filter holder is thoroughly flushed with sterile water or a different liquid, if required (buffer, physiological saline, peptone water, etc.). After flushing, the filter is incubated on a moistened nutrient pad.

If the sample to be tested has a viscous consistency, it can be diluted with a sterile solution using one of the above-mentioned liquids and, additionally, be warmed to make it easy to filter. Since the diluting solution is sterile, its volume does not have to be allowed for in calculating the CFU count.

In the case of solids, sample preparation mainly involves dissolving the sample. If this is not possible (e.g., for powder), the sample is thoroughly shaken in a rinse liquid to rub off the microbes from the surface of the granules or particles and thus to release them into the solution.

Samples with an oil base are no problem to filter. The only prerequisite that must be met is that both the filter support and the filter must be dry so that the pores of the filter cannot become completely saturated with water from below. After filtration, the filter must be rinsed using an emulsifying liquid to remove all traces of grease or oil. Then the emulsifier must be washed, in turn, with a rinse liquid.

Water-in-oil or oil-in-water emulsions, such as creams and ointments, pose considerable difficulties during sample preparation. These products, in particular, usually contain preservatives that can be most effectively removed by filtration. To prepare these samples for filtration, they must be suspended in an emulsifying agent (Tween 80 or 20 or Triton × 100), which must subsequently be removed by flushing.

2.5 Dimensions

Diameter of the nutrient pads:	50 mm
Thickness of the nutrient pads:	approx. 1 mm
Required volume of liquid for moistening:	3.5 ml
Diameter of the membrane filters:	47 or 50 mm
Diameter of the petri dishes:	56 mm
Height of the petri dishes:	13.5 mm

2.6 Materials

Petri dishes:	polystyrene
Pads:	cellulose
Membrane filters:	cellulose nitrate

2.7 Available Quantities

Standard package, ID letter "N":
100 nutrient pads in petri dishes +
100 membrane filters

Box dimensions:	35.2 × 15.5 × 12.3 cm
Weight:	1,188 g

Special package, ID letter "K":
25 polyethylene bags, each with
2 nutrient pads + 50 membrane filters

Box dimensions:	13.0 × 6.5 × 8.0 cm
Weight:	200 g

2.8 Formulas
The following Table lists the ingredients of the various types of Nutrient Pad Sets. The formulas are based on those commonly described in literature for agar and nutrient broth media, which are also used as reference media in testing our Nutrient Pad Set formulas for release.

Because of the variations in the cellulose pads used as support media, there may be slight variations in the amounts of the individual components.

Key to the letter codes in the Table on the next page for inhibitors and detergents as well as the dyes used.

Inhibitors and Detergents:

A. Sodium azide
B. Sodium sulfite
C. Tergitol
D. Bismuth-sulfite indicator
E. Sodium lauryl sulfate
F. Actidion
G. Gallic salt
H. Cetrimide
J. Sodium deoxycholate
K. Tween 80
L. Mixture of several inhibitors
M. Mixture of diff. inhibitors

Dyes:

a. TTC
b. China blue
c. Fuchsin
d. Bromothymol blue
e. Brilliant green
f. Aniline blue
g. Bromocresol green
h. Bromocresol purple
i. Phenol red
j. Rosolic acid
k. Neutral red
l. Crystal violett
m. MUG
n. Chromogen-mixture

Nutrient Pad Type Formula	Azide	Bismuth-Sulfite	Caso	Cetrimide	Chapman	Chromocult	ECD	Endo	Gluc-Try.	Lysine	Mac Conkey	Malt extract	MFC	Orange Serum	R2A	Sabouraud	Schauf.-Pot.	Standard	Standard TTC	Teepol	Tergitol TTC	Tomato Juice	VLB-S7-S	Weman	Wort	Yeast extract
Peptone	●	●	●	●	●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●			●	●
Meat extract		●			●											●					●					
Yeast extract	●							●					●	●	●		●	●	●		●	●	●			●
Malt extract																									●	
Liver extract																							●			
Beer wort																							●			
Orange serum														●												
Tomato juice																						●				
Dextrose | dextrin	●	●							●	●				●	●	●	●	●	●						●	
Lactose							●	●			●		●							●	●					
Saccharose																									●	
Maltose												●											●		●	
Mannitol					●																					
Gelatin																							●			
Starch																							●			
Glycerin				●																						●
Potassium phosphate	●						●	●						●	●		●			●					●	
Magnesium sulfate																	●					●	●	●		
Magnesium chloride				●																						
Nalidixic acid				●																						
Potassium sulfate				●											●											
Sodium chloride			●		●	●	●				●		●					●	●	●				●		
Iron sulfate		●																							●	
Ammonium chloride																										●
Sodium acetate																							●			
Calcium carbonate																								●		
Calcium chloride																								●		
Sodium phosphate		●																							●	
Sorbit						●																				
Sodiumpyruvate						●																				
Disodiumhydrogenphosphate						●																				
Sodiumdihydrogenphosphate						●																				
Potassium lactate										●																
Lactic acid										●																
Mixture of minerals										●																
Amino acids							●			●							●						●			
Malic acid																						●				
Vitamins										●							●						●			
L-lysine hydrochloride										●																
Tryptophan						●	●																			
Inhibitors and detergents	A	D		H		C E	G	B E J			G	G								E	C	F K	M	L		
Dyes	a	e			i	n	m	c	h		k l	f j					g		a	i	d		g	g		

2.9 Toxicity
All formula components that are classified as toxic are present in the finished nutrient pads in such low concentrations that they do not have to be declared according to the European Regulation 91/155 EEC.

2.10 Sterilization
Nutrient Pad Sets are sterilized by ETO, with the sterilization conditions depending on the type of Nutrient Pad Set and the special sensitivity of its culture medium.

ETO sterilization is carried out in a system that is operated with Sterisat gas. The Nutrient Pad Sets are degassed under controlled conditions. Following this procedure, no residual ETO can be detected (<= 1 ppm).

In-house validations are performed to ensure that after sterilization, the nutrients, active ingredients and inhibitors have their required activity, but that any resistant microbes that might have survived within the box or sublethally impaired microorganisms will not affect or interfere with growth on the filters of Nutrient Pad Sets.

2.11 Shelf Life
The shelf life of Nutrient Pad Sets is declared per lot. The expiration date is given on the labels of both the box and the interior packaging. The various types of Nutrient Pad Sets have different shelf lives, depending on their ingredients. The shelf life starting from the date of manufacture ranges between 9 and 24 months as follows:

24-month shelf life:
Malt extract, Sabouraud, Standard, Standard TTC, Wort.

18-month shelf life:
Orange Serum, Schaufus-Pottinger.

12-month shelf life:
Azide, Bismuth-Sulfite, Caso, Cetrimide, Chapman, Chromocult, ECD, Glucose-Tryptone, MacConkey, R2A, Teepol, Tergitol, VLB-S7-S, Weman, Yeast extract.

9-month shelf life:
Endo, Tomato Juice "Jus de Tomates," MFC.

Samples that have been filed for traceability are challenged with the relevant test organisms to check the selectivity and growth-promoting properties of Nutrient Pad Sets at regular intervals. This ensures that any changes in the material due to aging of the raw materials will not cause any problems with shelf life.

2.12 Filter Types Supplied with the Pads
Individually packaged and presterilized membrane filters of the pore size and color that are optimally suited to the particular application are included with each type of Nutrient Pad Set.

Filter Type	Pore Size	Color	Grid Color	For the Nutrient Pad Types:
13906	0.45 µm	White	Green	Cetrimide, Chapman, ECD, Endo, Glucose-Tryptone, MacConkey, MFC, Teepol, Tergitol, VLB-S7-S
13806	0.45 µm	Green	Green	Azide, Bismuth-Sulfite, Caso, Orange Serum, R2A, Standard, Standard TTC, Tomato Juice, Weman, Yeast extract
11406	0.45 µm	White	Black	Chromocult
13005	0.65 µm	Grey	White	Lysine, Sabouraud, Schaufus-Pottinger, Wort
13905	0.65 µm	White	Green	Schaufus-Pottinger
13004	0.8 µm	Grey	White	Malt extract, Orange Serum, Schaufus-Pottinger
13903	1.2 µm	White	Green	Schaufus-Pottinger

The effective filtration area of the disc filters is approx. 12.5 cm^2.
The flow rate depends on the particular pore size of the membrane filter.
For further information on Sartorius filters, please refer to the "Validation Guide for Sartorius Membrane Filters."

3. Test Parameters for Quality Assurance of Nutrient Pad Sets

3.1 Test Plan for Quality Assurance of Sartorius Nutrient Pad Sets

Test Plan/Test for	Sterility	Growth	pH	Inhibitors	Shelf Life
Incoming inspection	+	+	+	+	–
In-process control	–	–	+	–	–
Final inspection and testing	+	+	+	+	+
When performed	For each lot	For each lot	For each lot	For each lot or delivery	Regularly

3.2 Test Methods in Detail

3.2.1 Sterility

3.2.1.1 Incoming Inspection
The sterility of the petri dishes is tested at regular intervals.

A defined number of petri dishes are each filled under sterile conditions with 10 ml of Soybean-Casein Digest Medium and incubated for 5 days.

Criterion for release: All test petri dishes must be sterile.

In addition, the sterility of the petri dishes is tested along with the other components of the Nutrient Pad Sets during sterility testing of each lot as part of final inspection.

3.2.1.2 Final Inspection and Quality Control Testing
Ten test nutrient pads of each lot are moistened in their original plates (petri dishes) with 8 ml of sterile water and incubated at 30°C for 5 days. Afterwards, the test nutrient pads are examined for signs of turbidity.

Criterion for release: All 10 test nutrient pads must be sterile after incubation.

3.2.2 pH

The aqueous extract of 10 test nutrient pads from each lot is measured with a pH meter (manufacturer: WTW) previously calibrated using 2 buffers.

Criterion for release: The pH must be within the stringent in-house tolerance limits; see Table in Section 2.2, page 7.

3.2.3 Growth

3.2.3.1 Test Equipment
Stainless steel vacuum filter holder with 40 ml funnel capacity, Sartorius order no. 16220

Suction flask, 2000 ml,
Sartorius order no. 16672

Laboratory vacuum pump,
Sartorius order no. 16612 (220 V)
or 16615 (110 V)

Midisart 2000, Sartorius order no. 17804, used as a moisture barrier between the pump and suction flask

Dosing syringe,
Sartorius order no. 16685-2

Minisart syringe filter holders,
Sartorius order no. 16534 K

Incubator,
Sartorius order no. 17645 (220 V)

Test organisms:
see Table in Section 3.2.3.2

3.2.3.2 Method
A test suspension is prepared from the test organisms. This suspension has a density of approx. 10 microbes per ml. Ten samples of this suspension are filtered through each of the membrane filters supplied for the various Nutrient Pad Set types. Afterwards, each filter is flushed with 10 ml of sterile water and placed on each Nutrient Pad, which has been moistened with 3.5 ml of sterile water. Agar plates (manufacturer: Difco, Merck or Unipath) comparable to each Nutrient Pad Set are used as controls.

The incubation temperature and time depend on the particular requirements of the type of Nutrient Pad.

Criteria for release:
The CFU count on the Nutrient Pad must be at least 90% of the count on the comparable agar plate. The colonies must exhibit the typically expected growth pattern in terms of shape and color and must have the other properties as those indicated on the reference agar plate. For an exact description of these growth patterns, refer to the Section "Description and Growth Evaluation Samples" given in the brochure "Microbiological Testing of Foods, Beverages and Pharmaceuticals" that is available free on request from Sartorius.

During development of Sartorius Nutrient Pad Sets, the growth-promoting properties of the individual types were tested with a large number of test organism strains. The most important strains are re-used at regular intervals to ensure the selectivity and growth-promoting properties of each Nutrient Pad Set type. For routine testing of lots for release, only selected test organisms that are highly fastidious in terms of nutrients are inoculated.

Table: Test Organisms and Which Are Used for Testing Nutrient Pad Set Types

Test Organism	ATCC No.	Nutrient Pad Type
Aspergillus niger	16404	Caso, R2A, Sabouraud
Bacillus cereus	11778	MacConkey, Orange Serum, Tergitol
Bacillus stearothermophilus	7953	Glucose Tryptone
Bacillus subtilis	6633	Bismuth Sulfite, Caso, Glucose Tryptone, Schaufus Pottinger, Standard, Standard TTC, Yeast extract
Brevundimonas diminuta	19146	Cetrimide
Candida albicans	10231	Caso, Malt extract, Lysine, Orange Serum, Sabouraud, Schaufus Pottinger, Wort, R2A
Enterobacter aerogenes	13048	ECD, Endo, MFC, Teepol, Tergitol, Chromocult
Enterococcus faecalis	29212	Azide, Caso, Chapman, Standard, Standard TTC, Yeast extract
Enterococcus faecium	35667	Azide, MacConkey
Escherichia coli	8739	Azide, Bismuth Sulfite, Caso, Cetrimide, Chapman, Chromocult, ECD, Endo, Glucose Tryptone, MacConkey, MFC, Standard, Standard TTC, Teepol, Tergitol, R2A, Yeast extract
Klebsiella pneumoniae	13883	Endo, MFC, Teepol, Tergitol, Chromocult
Lactobacillus lindneri	20690*	VLB-S7-S, Tomato Juice
Lactobacillus plantarum	14917	VLB-S7-S
Leuconostoc mesenteroides	8293	Tomato Juice (Jus de Tomates), Weman
Oenococcus oeni	23279	Tomato Juice (Jus de Tomates), VLB-S7-S
Mixed culture from honey		Weman
Mixed culture from raw sugar		Glucose Tryptone, Weman
Mixed culture from tap water		Caso, Glucose Tryptone, Standard, Standard TTC, Yeast extract, R2A
Pediococcus damnosus	29358	VLB-S7-S
Penicillium commune	10428	Lysine, Orange Serum, Sabouraud, Schaufus Pottinger, Wort, Malt extract, Wort
Proteus mirabilis	14153	Bismuth Sulfite, Cetrimide, Chapman, ECD, Endo, MacConkey, MFC, Teepol, Tergitol
Pseudomonas aeruginosa	9027	Azide, Cetrimide, ECD, R2A
Rhodotorula mucilaginosa	70404*	Lysine, Orange Serum, Sabouraud, Schaufus Pottinger, Wort, Malt extract
Saccharomyces cerevisiae	9763	Lysine, Orange Serum, Sabouraud, Schaufus Pottinger, Tomato Juice (Jus de Tomates), Wort, Malt extract
Salmonella cholerasuis	8293	Bismuth Sulfite, Endo, MacConkey, Chromocult
Staphylococcus aureus	6538P	Azide, Bismuth Sulfite, Caso, Cetrimide, Chapman, ECD, MacConkey, MFC, Standard, Standard TTC, Teepol, Yeast extract
Staphylococcus epidermidis	12228	Chapman

* DSM-no.

3.2.4 Growth Inhibition Test

Sartorius, the manufacturer, takes samples of each lot of absorbent pad material supplied. This material is released for use in Nutrient Pad Set production only if it is guaranteed to be free of inhibitors in addition to complying with the technical specifications. The growth inhibition test is used to check whether this material is free of inhibitors.

3.2.4.1 Test Equipment

Petri dishes,
Sartorius order no. 14311-60 N
Membrane filters,
Sartorius order no. 13906-50 ACN
Culture medium: Caso broth,
Sartorius order no. 14162 K
Filtration equipment and incubator as listed in Section 3.2.3.1
Test solution: Suspension of Pseudomonas aeruginosa (see list in Section 3.2.3.2) and a mixed culture from tap water.

3.2.4.2 Method

Ten samples are taken from each roll of absorbent pad material and sterilized with ETO. Ten reference samples of a released lot are used as controls. The test samples are placed in sterile petri dishes under sterile conditions, and each is moistened with 3.5 ml of sterile Caso broth. The test suspensions described in Section 3.2.3.2 are each passed through a membrane filter. Five test samples are inoculated with the Pseudomonas aeruginosa suspension and 5 with a mixed culture from tap water. The same is done for the reference samples.

Criterion for release:
The CFU count and the growth pattern of the test samples must match those of the reference samples. No growth inhibition may be visible.

3.2.5 Weight per Unit Area, Thickness and Absorbency

These technical specifications are defined on our in-house Specifications Sheets and are checked by the Sartorius Incoming Inspection Department.

Criteria for release:
Thickness of the pad material:
1 mm +/– 20%

Weight per unit area: 360 g/m^2

Absorbency: 3.0 ml demineralized water must be absorbed

3.2.6 Reagents

To check the quality of the reagent lot used, a sample lot of a Nutrient Pad Set type is manufactured in which this reagent is contained in the culture medium formula. The corresponding agar media lot supplied by a well-known, reliable manufacturer are used as controls.

Criterion for release:
Both the growth pattern and the CFU count of the sample lot must match those of the reference lot.

4. Reproducibility

The reproducibility of the results obtained with Nutrient Pad Sets is demonstrated by performing numerous comparative tests of each lot and the corresponding reference agar plates as a regular part of QC inspection for release of new lots. The data given in this Validation Guide for Standard, Wort and Endo Nutrient Pad Sets along with the most important test organisms are representative examples for the variety of the Nutrient Pad Set types tested.

Nutrient Pad Set Type	Lot No.	Test Organism	ATCC No.	DSMZ No.	Growth vs. That on Reference Agar in %
Endo NKS	020414	Escherichia coli	8739	1576	103.5
	020419	Escherichia coli	8739	1576	98.3
	020487	Escherichia coli	8739	1576	100.5
Endo NKS	020419	Escherichia coli	25922	1103	100.7
	020484	Escherichia coli	25922	1103	100.5
	020485	Escherichia coli	25922	1103	102.6
Wort NKS	020421	Sacch. cerevisiae	–	1334	98.7
	020459	Sacch. cerevisiae	–	1334	95.0
	020475	Sacch. cerevisiae	–	1334	101.6
Standard TTC	020460	Escherichia coli	8739	1576	103.4
	020462	Escherichia coli	8739	1576	99.8
	020472	Escherichia coli	8739	1576	105.8
Standard TTC	020460	Mixed culture from tap water	–	–	104.0
	020471	Mixed culture from tap water	–	–	102.4
	020504	Mixed culture from tap water	–	–	103.9

5. Comparative Tests

In comparative tests, it was confirmed that the number, shape and color of the colonies encountered on the membrane filters of the Nutrient Pad Sets tested were comparable to those on the control agar plates and on the absorbent pads moistened with culture media. The products of various well-known manufacturers were used in these test studies. Excerpts of the reports on these studies can be requested from Product Management, Laboratory Business Unit, of the Sartorius Separation Technology Division in Germany.

6. Regulations

The compositions of the pads are based on the recommendations of numerous different standards and regulations. Some of them are mentioned here as an example.

Regulation	NKS Type
DAB	Caso (Tryptic Soy), Cetrimide, MacConkey, Sabouraud
USP	Bismuth-Sulfite, Caso, Cetrimide, Chapman (Mannitol Salt), MacConkey, Sabouraud, Teepol
APHA	Azide, Bismuth-Sulfite, Caso, Cetrmide, Chapman, ECD, Endo, Glucose-Tryptone, Malt extract, McConkey, MFC, Orange Serum, R2A, Sabourand, Standard, Standard TTC, Yeast extract
TVO, MTVO	Azide, Caso, Cetrimide, ECD, Tergitol TTC, Yeast extract
Standard Methods	Azide, Caso, Endo, MFC
LMBG	Azide, ECD, Endo, MacConkey

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