5. Services

Introduction

PAN-Biotech offers you a variety of services and test procedures for your products.

The following services and test procedures are available for you:

- Special Processing of Serum lots
- Biochemical Tests
- Tests for pathogenic Agents (Bacteria, Viruses, Fungi)
- Functional Tests
- Cell Processing
- Special Services

We deliver these services fast, cost-effective, using the latest up-to-date techniques and in the highest quality for you. You can profit from our expertise!

If you need further special tests or particular services let us speak about it. In most cases we can find a solution for you.

Special Processing of Serum Lots

Sterile filtration
The sterile filtration is performed via a validated process. The raw material passes a series of filters with decreasing pore sizes. The last filtration step is done with a 0,2 µm pore size sterile filter.

IgG extraction
With a chromatographic method (affinity chromatography) the antibodies in the serum are near-completely removed (< 5 µg/ml). The biological activity of the serum is not affected.

Active charcoal filtration
Serum is heated in a water bath with dextran and activated charcoal. The activated charcoal, together with the substances bound up in it, is then removed by centrifugation and filtration.

Dilipidisation
Lipids are removed from serum by affinity chromatography.

High-grade dialysis
Serum is dialysed whit a 10,000 dalton exclusion membrane against a physiological saline solution.

Heat inactivation
Serum is heated for 30 minutes at 56 °C in a water bath, whereby it is shaken softly several times.

Gamma irradiation
Serum is exposed to irradiation with at least 25 kGy.

Biochemical Tests

Protein
Colorimetric test (Biuret-reaction). Bivalent copper reacts in alkaline solution with the peptide bond of albumin to the characteristic purple coloured biuret complex. With sodium-potassium-tartrat a precipitation of copper-hydroxide and with potassium-iodide the automatic reduction of copper is prevented. The colour intensity is directly proportional to the albumin concentration, which is analyzed photometricly.

Osmolality
The osmolality is analyzed by freezing point depression. Calibrating the osmometer is made by the use of standard solutions.

pH-value
Assessed with pH-electrode.

Haemoglobin
The concentration of haemoglobin is assessed spectrophotometricly with three different wave lengths.

IgG
Radial immune diffusion. Antibodies in agarose precipitate in the equivalence area with antigens, which, pipetted into a gel hole, diffuse radial outwards. The diameter of the precipitation ring is proportional to the concentration of the applied antigen containing solution.

Biochemical Tests

Triglyceride
Enzymatic colour test on the basis of the Trinderreaction with extinction increase.

Cholesterin
Enzymatic colour test with extinction increase.

Glucose
Enzymatic colorimetric test on the basis of the Trinderreaction.

Tetracyclin
Sera are tested for suitability for tet-inducible systems. If a tet-off-system is used, an acceptable expression of aim proteins is only warranted when no tetracycline is attended. Necessary conditions therefore are cell culture media and sera containing no tetracycline. For the tests a CHO cell line is used, which contains a tet controlled luciferase gen (CHO-Luc) in a reporter gen construct. When these cells are cultured without tetracycline, an expression of the luciferase enzyme will be induced, which is quantified with Promegas luciferase test system.

Endotoxin
Endotoxins are an essential part of the lipopolysaccharides of the exterior cell wall of gram-negative bacteria. The endotoxin value is analyzed with the kinetic LAL method. The limulus amoebacytes lysate (LAL) consist of an aqueous extract of horseshoe crab (limulus polyphemus) blood cells. In the presence of endotoxin LAL generate a turbidity where with the endotoxin value of a sample can be analyzed.

Tests for Pathogenic Agents

Mycoplasma
Mycoplasmas are the smallest self breeding prokaryotes. As they have no cell wall the form of the mycoplasmas is very variable and they can pass the usual sterile filter (0.2 µm). Contaminations of cell cultures with mycoplasmas are abundant and not easy to discover, because they do not always cause significant effects.

The following detection systems are used :

- **Microbial culture.** After concentration in special media under aerobic and anaerobic conditions the assays are plated on agar plates. After additional incubation steps a microscopic analysis take place. Mycoplasma contamination is identifiable by the fried egg form of the colonies.
- **DNA-binding fluorescence colorant (DAPI, bisbencimide).** Contaminations can be detected by colouring the mycoplasma DNA with special binding fluorochrome DAPI (4-6-diamidino-2-phenylindol-di-hydrochloride). Under the fluorescence microscope the mycoplasmas appear as equally formed, small, bright shining points or accumulations of them outside the indicator cells (often at the cell membrane). As the mitochondrial DNA is coloured only marginally, the background fluorescence is quite small.
- **Detection of mycoplasma specific enzymes.** With special test kits mycoplasma specific enzymes are detected.
- **PCR ribosomal RNA.** Amplified mycoplasma specific rRNA segments are separated and detected electrophoreticly.

Virus tests according EMEA guidelines
The following virus tests are performed according to the EMEA guideline CPMP/BWP/1793/02 (note for guidance on the use of bovine serum in the manufacture of human biological medicinal products) :

- Bluetongue and related orbi viruses
- Bovine adenovirus
- Bovine parvovirus
- Bovine respiratory syncytial virus (BRSV)
- Bovine viral diarrhoea virus (BVDV)
- Rabies virus (rabies)
- Reo virus
- Bovine polyoma virus (BPyV)

Sterility
The absence of bacterial or fungal contaminations is verified by incubation with Caso-Bouillon or Thioglycolat-Bouillon according to Pharm. Eur.

Bacterial count
The detection of the total number of viable aerobic germs will be either done by membrane filtration or plate-flushmethod or as surface-method. The microorganisms are detected as colony building units per ml (CBU/ml) on casein peptone-soy flour peptone-agar plates.

Functional Tests

Plating efficiency
To get the plating efficiency, murine fibroblasts (L929) are plated into a Petri dish with 20 % serum and DMEM as basal medium in a very small density (500 cells/dish). After an incubation period of 14 days in an incubator at 37 °C and 5 % CO_2 fumigation, the cell colonies are counted after Giemsa coloration (total plating efficiency). The results are normalized against a further tested reference serum (relative plating efficiency).

Cloning efficiency
The cloning efficiency shows the ability of a serum lot to support the cloning and the growth of murine myeloma cell line and derived hybridoma lines. Therefore SP2/0- Ag14 cells (murine myeloma) are plated on microtiter plates (one cell per well) with 20 % serum and RPMI 1640 as basal medium in a very small density. After 7 days in an incubator at 37 °C and 5 % CO_2 fumigation the cell colonies are counted (total cloning efficiency). The results are normalized against a further tested reference serum (relative cloning efficiency).

Growth test
In this assay the ability of a serum lot to support the proliferation of murine fibroblasts (L929) and murine myeloma cells (SP2/0-Ag14) is searched. The cells are plated at a relative low density of 1.000 cells/ml for SP2 and 10.000 cells/ml for L929. After an incubation at 37 °C and 5 % CO_2 fumigation cells are counted in a metering chamber on the second, fifth and seventh day. A control culture is carried along the test.

Cell Processing

Establishment and expansion of special cell cultures
Primary human cells from many different tissues are isolated and cultured under the required conditions.

Development of cell specific media for unique applications
Serumfree and proteinfree media (growth media, differentiation media) are developed and optimized for many different cell lines and primary cells according to the special application.

Purchasing and processing of special cells
Isolation, cultivation and incubation of special cells according to the customer‘s request.

Special Services

There are currently only a few, manually very complex and expensive systems available for complex in-vivo simulation problems. For special areas in pharmaceutical and biotechnology research there is a high need for appropriate in-vivo near and automated cell systems and cellular applications.

To address this demand, which is only met in part at the moment, each of the PAN development departments with its specific areas of expertise (media, cells, automation equipment) and still developed several applications for the automated in-vivo-near cellular tests which come together in the "In-Vitro-Man" concept to create suitable solutions.

Several applications are suitable for special experimental settings in medicine, oncology and regenerative medicine which are focused in the "In-Vitro-Man concept.

PAN offers the following concepts as services for customers which are performed in PAN s labs or PAN implements theses services in the customers labs as fee-for-service projects.

- Cellular, endothelial and myocardial stem cell models under physiological flow and pressure conditions with defined shaer-rates and shear-stress to simulate the conditions of vessel repair and regeneration in cardiovascular conditions.
- Cellular, automated spheroid systems for individual patient testings of the effectiveness of cytostatic drugs for tumor therapy and individual therapy optimization.
- Cellular and automated tumor models for research into tumor angiogenesis, in order to derive appropriate therapy options and to screen and evaluate candidate agents or agent principles for inhibiting tumor angiogenesis.
- Cellular models for the investigation of cellular interactions between patient s cancer cells and primed/ activated immune cells (e.g. dentritic cells, cytotoxic T-Lymphocytes) after immunological activation or cancer vaccination.
- Cellular models for the investigation or identification of tumor cells under special conditions (semipermeable membrane coated with endothelial cells/HUVECs) to investigate the tumor migration through endothelium.

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