Process Plant and Equipment

VDMA

Information sheet

Riboflavin test for low-germ or sterile process technologies

Fluorescence test for examination of cleanability

For food, aseptic, pharmacy and chemistry

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Contents

This publication has been prepared by the "Riboflavin Test" Working Party of the Sterile Process Engineering Group of VDMA. It is available as a downloadable file under www.vdma.org/verfahrenstechnik. Suggestions for improvements and additions can be sent to the address below.

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1. Introduction

Tests for examination of cleanability play a major role in sterile process technology. A variety of tests are applied in practice, depending on the respective use case, suitability or requirements. This information sheet does not specify further details in this respect. The described fluorescence test is first and foremost suitable for the components named in the scope, as far as these can be examinated by means of visual inspection. This being the case, the fluorescence test is not intended to replace other well-established tests, but rather to supplement available possibilities in this sector.

The objective of this information sheet is to provide manufacturers, suppliers and users with a document that can simplify the accord, planning, carrying out and documentation of a fluorescence test. In doing so, the information sheet summarizes different tests commonly used in practice as well as comprehensive experience gathered with these tests to provide a possible coarse of action. **Manners of procedure or accords that deviate in part or completely are, however, expressly possible.**

2. Scope

The scope of this information sheet covers components, apparatuses, machinery and plants (also referred to in this information sheet as 'components') for low-germ or sterile process technologies with high or highest requirements regarding cleanability, as far as these are accessible for visual inspection. These components are used in the food, aseptic processing, pharmaceutical and chemicals sector, e.g. vessels, reactors, filter equipment, pumps, agitators, centrifuges, pasteurizers, filling systems etc. including fittings and peripheral equipment.

Note on use:
This information sheet gives advices to the user. It is, however, incumbent upon the user of the information sheet to verify or consider requirements, the current validity thereof and necessary measures concerning the user's concrete use case. This concerns in particular all laws, ordinances, directives etc. that could be relevant for the respective case of use.

3. Terms, definitions

- **Fluorescence test**
  Test using a fluorescent substance for examination of cleanability of components
- **Cleanability**
  Complete removal of the test solution by the cleaning medium **under application of the selected conditions** with regard to the cleaning elements, cleaning process or the design of the component
- **Cleanability test**
  Test for complete cleanability under the conditions selected for the fluorescence test
- **Weak point test**
  Test for localizing critical points;
  Usually parameter values of the cleaning procedure are used which deviate from those of the cleanability test (reduced pressure or throughput of the cleaning medium or duration of cleaning process)
- **Optimization test**
  Stepwise optimization and testing of the suitability of new parameter values through separate, new cleanability tests
- **Test solution**
  Solution for carrying out the fluorescence test
- **CIP cleaning**
  Cleaning of components in assembled condition (Cleaning In Place)
- **Cleaning water**
  Water for cleaning the component being examined
- **Fully demineralised water**
  Fully desalinated water;
  also referred to Aqua purificata (AP) or Purified Water

- **WFI water**
  Water For Injection
- **Critical points**
  Points that are difficult to clean and can be cleaned completely
- **Non-critical points**
  Points that are easy to clean and can be cleaned completely
- **Non-cleanable points**
  Points that cannot be cleaned completely
- **Cleaning element**
  Element for targeted application of cleaning liquid to the component to be examined; Examples of cleaning elements: spray ball, rotating jet cleaner, cleaning nozzle, spray lance
- **Surfaces to be examined**
  Areas of the component being examined that are to be accounted for in the fluorescence test
- **Surfaces to be wetted**
  Surfaces to be examined on which the test solution is to be applied
- **Carrying out the test**
  Application and removal of the test solution as well as the subsequent inspection for remaining fluorescence
- **Workplace limit value**[1]
  Limit for the time-dependent average concentration of a substance in the air at the workplace, in relation to a given reference period.

4. Aim of the fluorescence test

The fluorescence test described in this information sheet is for the examination of cleanability. This is carried out by the examination of the basic accessibility to, as well as the complete wetting of all areas in which a verification of cleanability through the cleaning medium is required.

The cleanability test is aimed to verify complete cleanability; the result of the test is a **qualitative statement**. **In addition to this,** the step-by-step or repeatedcarrying out of the fluorescence test also enables qualitative statements or examination of measures for improving or optimizing the cleaning process. Table 1 specifies the goals that can be achieved with the fluorescence test:

Fluorescence test:	Aim of the test:	Criterion of quality after the test:
Weak point test	Localizing critical points; provided as optional preliminary stage to the cleanability test.	- Visible fluorescence[2] at critical points (acc. definition in Clause 3); these are to be confirmed through a cleanability test.
Cleanability test	Verification of full cleanability.	- No visible fluorescence[2].
Optimization test	Stepwise optimization and checking of suitability of new parameter values through separate, new cleanability tests.	- No visible fluorescence[2]. - Improved parameter values (e.g. reduced water consumption, shorter cleaning time)

Table 1: Achievable goals using fluorescence test acc. to information sheet

[1] Specifies the concentration of a substance at which acute or chronical health implications are generally not to be expected. Definition from Hazardous Substance Ordinance of December 23, 2004 (BGBl. (German Federal Law Gazette)I P. 3758, 3759), last amanded through Article 4 of the Ordinance of March 6, 2007 (BGBl. I P. 261)
[2] When checking the surfaces being examined for any fluorescence by means of visual inspection using a UV lamp.

5. Instatallation, equipment, specifications and carrying out the test

5.1 General notes and points to be observed

Regulations and directives relating to occupational health and safety must always be observed when carrying out the test. Furthermore, special reference is made to the following:

- Testing personnel:
  No specific requirements are placed with regard to the education of testing personnel. Testing personnel should, however, be suitably and trained to carrying out the test or guided by in-house work instructions.
- UV lamp:
  The use of a UV lamp can cause damage to eyes through penetrating UV rays. It is therefore necessary to wear safety goggles and to observe any additional protection measures specified by the manufacturer of the UV lamp.
- Occupational safety:
  As the UV lamp is always used in a moist environment personal fuse protection of the electrical supply should be provided, e.g. using isolating transformers[3]. If it is necessary to light up a vessel with a UV lamp when carrying out the test and to enter a vessel, this precautionary measure is strongly advised.
- Degreasing agent:
  The safety data sheet of the supplier must be available and must be observed.
- Fluorescent substance:
  The safety data sheet of the supplier must be available and must be observed.
- Drying out:
  When completely dried there is no homogeneous thickness of the applied layer of fluorescent substance. Reproducible verification of the cleanability is not possible in this case. With partial or incomplete drying there is also no reproducible condition with regard to the removal or dissolving of the fluorescent substance. Drying out of the test solution must therefore be avoided.

5.2 Test build-up

The test build-up for performing a fluorescence test can be carried out in compliance with the arrangement shown in Annex 8.2, Fig. 1.

5.3 Test equipment and specifications

5.3.1 Test solution

A test solution has to be prepared prior to carrying out the test. Table 2 in Annex 8.1 specifies Ingredients and recipes of test solutions . These ingredients and recipes have proved their worth in fluorescence tests in practice and in trial carried out during the preparation of this information sheet.

It is basically also possible to use other partially or completely deviating ingredients or recipes for the test. Deviations and the effect these may have are to be taken into account or arranged separately, if necessary.

Note on recipes containing ethanol: Some recipes are used which require the addition of ethanol in water for the preparation of test solutions (for improved wettability, but also increasing the drying tendency). This can have an impact on the protection against explosion, occupational health and safety and must be considered separately, if necessary.
According to calculations and assuming realistic conditions, it must be expected that the limit value for the workplace will be exceeded; the ethanol intake through inhaled air can cause a significant increase in the blood alcohol level (allowing for typical vegetative physiological values for breathing rate etc.). Under realistic temperatures it may also occur that the lower ignition limit of the ethanol air mixture in the gas phase is exceeded.
If necessary, effects resulting from the use of recipes containing ethanol are to be accounted for through own observations, measurements and/or appropriate measures.

[3] Isolating transformers transform applied electrical line voltages in the ratio of 1:1 to a winding with safe electrical separation (increased or doubled isolation to the system). They generate a non-earthed, free potential of the output voltage so that no current can flow through the body to earth upon contact. They are used for works on devices fed with line voltage to reduce the hazard of an electric shock.

5.3.2 Water used to prepare the test solution

The quality of the water used for preparing the test solution should have at least the same quality as that of the cleaning water (see Clause 5.3.3). To avoid deposits of minerals such as lime, demineralized water should always be used as a minimum quality for preparing the test solution. The water for the test solution should be at room temperature.

5.3.3 Cleaning water

Water of at least drinking water quality is to be used as cleaning water. The temperature of the cleaning water is to be in the range of 12 – 25 °C. At temperatures lower than this, a decline in the cleaning result is to be expected.

5.3.4 Darkening

It must be possible to darken the area of the surfaces to be examined; this only applies if the surfaces to be examined are not automatically in the dark due to their arrangement (e.g. on the inside of vessels).

5.3.5 Inspection lamp (UV lamp)

A UV lamp is used to make the fluorescence of the test solution visible, safety notes in this respect are given in Clause 5.1. The common wavelength for UV lamps used for the fluorescence test is 365 nm.

5.3.6 Surfaces to be examined

Surfaces for exmaninations are usually the inside surfaces of a component being examined including fittings and, wherever cleaning elements are available for cleaning outer surfaces, also the corresponding outer surfaces.

Note: To save time, it may be expedient when testing large, interconnected surfaces, not to wet all parts with the test solution. This can be the case, for example, with parts of large, interconnected surfaces of a vessel wall, as long as it can be assumed that these surfaces will react in the same way as the adjacent, fully wetted parts when cleaning off the test solution.
Surfaces not to be wetted are still to be attributed to the surfaces to be examined and accounted for in carrying out the test and documentation.

If parts of the surface to be examined are not to be wetted with test solution, this is to be arranged in advance and documented before carrying out the test.

5.3.7 Pre-cleaning

The surfaces to be examined must look clean and and be grease free.

Note: If due to the design or operation it is not possible to make the surface completely grease free it must be taken into account that at these points there will be reduced adherence and consequently easier removal of the test solution. The wettability of these areas can therefore not be evaluated using the fluorescence test.

5.3.8 Adjustment of components

The component to be examined, e.g. a vessel or piece of equipment must be positioned as instructed. Any deviations are to be corrected or documented if necessary.

5.3.9 Spray balls/nozzles and fittings

Spray balls/nozzles in or on the component to be examined must be mounted in compliance with the specification (e.g. shop drawing, assembly instructions) of the component to be examined. For the cleaning process all fittings required for the operation must be installed.

5.3.10 Pressure and flow rate measurement

Prior to every connection of a spray ball/nozzle a pressure and flow rate measurement should be carried out (ideally required as standard). If this is not possible, the conditions at the individual spray balls/nozzles must be calculated using the available data. The number, position and arrangement of pumps, pressure and flow rate measurements and spray balls/nozzles should therefore be outlined. (comp. Clause 7.1).

5.3.11 Cleaning procedure

The cleaning procedure for the test is carried out using cleaning water (see Clause 5.3.3). The duration of the actual cleaning process has to be adjusted to the actual degree of contamination during the later use.

The cleaning procedure is usually specified by the supplier of the component being examined (e.g. complete vessel, equipment etc. including fixtures in compliance with Clause 2). In doing so, the aim of the fluorescence test (see Clause 4, Table 1) is to be observed. The specification for the cleaning procedure should contain details on

- duration,
- pressure,
- flow rate and
- sequence

of the cleaning element application. It should also contain details on

- filling levels of the component and
- valve positions as well as
- positions and/or movement/speed of rotation of the component's active elements.

A suitable cleaning procedure may involve the application the cleaning elements or element-free connections with water, or the movement/speed of rotation of moving elements in or through cleaning water. Beyond this, an appropriate cleaning procedure can also comprise a random combination thereof.

5.4 Carrying out the test

Notes:
Before carrying out the test it is important to observe the points described in Clauses 5.1, 5.2 and 5.3. The documentation prior to performance of the test is described in Clause 7.1.

Carrying out the test:

1. Apply test solution to surfaces to be wetted using an atomizer nozzle. The surfaces to be wetted must be wetted completely.
   As an alternative the surfaces to be examined can also be wetted with test solution through flooding and subsequent emptying of the component being examined. When flooding, always make sure that the component can be completely flooded.

2. Bring component to be examined into correct operating condition.

3. Carry out the cleaning procedure described in Clause 5.3.11.

4. Visually inspect the surfaces to be examined for detectable fluorescence using a UV lamp.

6. Evaluation of the fluorescence test

The fluorescence test is considered as successfully passed when the criterion of quality described in Table 1 is met after completion of the test.

If the **cleanability test** for the component being examined is failed, the cause of this failure must be determined. The test is then repeated after correction measures have been carried out (e.g. modification of the cleaning procedure) and possibly coordination with the client. The new test conditions must be documented.

When carrying out the **weak point test** it may occur that it is not the associated criterion of quality that is met, but rather that of the cleanability test. In this case, it is recommended to mutually acknowledge the weak point test as cleanability test.

7. Documentation of the fluorescence test

The following listed items are to be documented:

7.1 Documentation of fluorescence test before carrying out the test

a) Test build-up
   - o Description, sketches/shop drawings, or pictures of the installation
   - o Wavelength of used UV lamp

b) Component to be examined
   - o Designation of the component to be examined
   - o Drawing number (possibly revision number) of the component to be examined
   - o Serial number or factory number of the component to be examined
   - o Just when some parts are not to be wetted with test solution: surfaces to be examined (comp. 5.3.6; if necessary using drawings or sketch diagrams to specify)

c) Measuring devices and reference measuring devices used for the test
   - o Name and test equipment number of the used reference measuring devices
   - o Calibration protocols of the used measuring equipment

d) Test solution/cleaning water
   - o Quality of water used to prepare test solution
   - o Temperature of water used for preparing test solution (room temperature: yes/no)
   - o Recipe of the used test solution (see Annex, Clause 8.1)
   - o Quality and temperature of cleaning water (see Clause 5.3.3)

7.2 Documentation of fluorescence test during carrying out the test

a) Description of test in compliance with Table 1 (e.g. "weak point test")
b) Cons. number of test
c) Date, test begins (time of day)
d) Confirmation 'test solution fluorescing'
e) Confirmation that surfaces to be wetted have been completely wetted with the test solution, or the component has been completely filled with test solution
f) Confirmation 'component is in correct operating condition'
g) Start of cleaning procedure
h) Application of cleaning elements, if applicable:
   - o duration
   - o pressure
   - o flow rate
   - o sequence
i) Filling levels of the component, if applicable
j) Valve positions, if applicable
k) Positions and/or movement/speed of rotation of active elements of the component, if applicable
l) End of cleaning procedure
m) Carrying out and result of visual inspection using a UV lamp

7.3 Documentation of the fluorescence test after carrying out the test

a) Date, end of test (time of day)
b) Position and shape of critical points, if applicable
c) Pictures of condition of the component being examined after completion of the test (optional)
d) Evaluation according to Clause 6

8. Annex

8.1 Ingredients and recipes of test solutions

Recipe No.:	1	2	3
Case of application:	All components	Only components that are completely filled with test solution (flooded).	Test for design experiments, where critical points are difficult to define
Remark:	Application of the test solution can already be identified with the naked eye, solution has been sprayed on evenly; drying of the test solution (compare Clause 5.1) is avoided.	Manual application of the test solution is obmitted. Addition of hydroxyethyl cellulose is therefore also no longer necessary. With this recipe partially thinner layer thicknesses occur when applied by spraying. These layers are not adequately identifiable with the naked eye, could lead to incorrect conclusions in the assessment of the cleaning result. The test solution is therefore only to be used for complete flooding.	Increased adhesion of the test solution. Appropriate for cases in which there is only a narrow margin between adhesion and removal of the test solution.
Constituent / Addition			
Riboflavin (dyes, increases viscosity and is fluorescent); CAS-No.: 83-88-5	0.2 g	0.2 g	1 g
Water (serves as solvent and carrier medium of the riboflavin); For requirements see Clause 5.3.2.	1000 ml	1000 ml	1000 ml
Hydroxyethyl cellulose ("HEC", for increasing viscosity and layer thickness); Requirements: normal type (not allyl modified), with swelling delay (reacts with delayed swelling), viscosity class: 100 000 mPas (in 1.9 % solution, 20 °C, 20 °GH); Viscosity of applied HEC test solution: 50 -75 mPas	Recommended (not absolutely essential): 5 g	-	10 g

Table 2: Ingredients and recipes of test solutions

Note:
Instead of riboflavin also uranin (CAS-No.: 518-47-8) can be used as fluorescent substance. With the same dosing, test solutions of both fluorescent substances are equally good when applied in the fluorescence test.

8.2 Schematic sketch of an installation for carrying out a fluorescence test

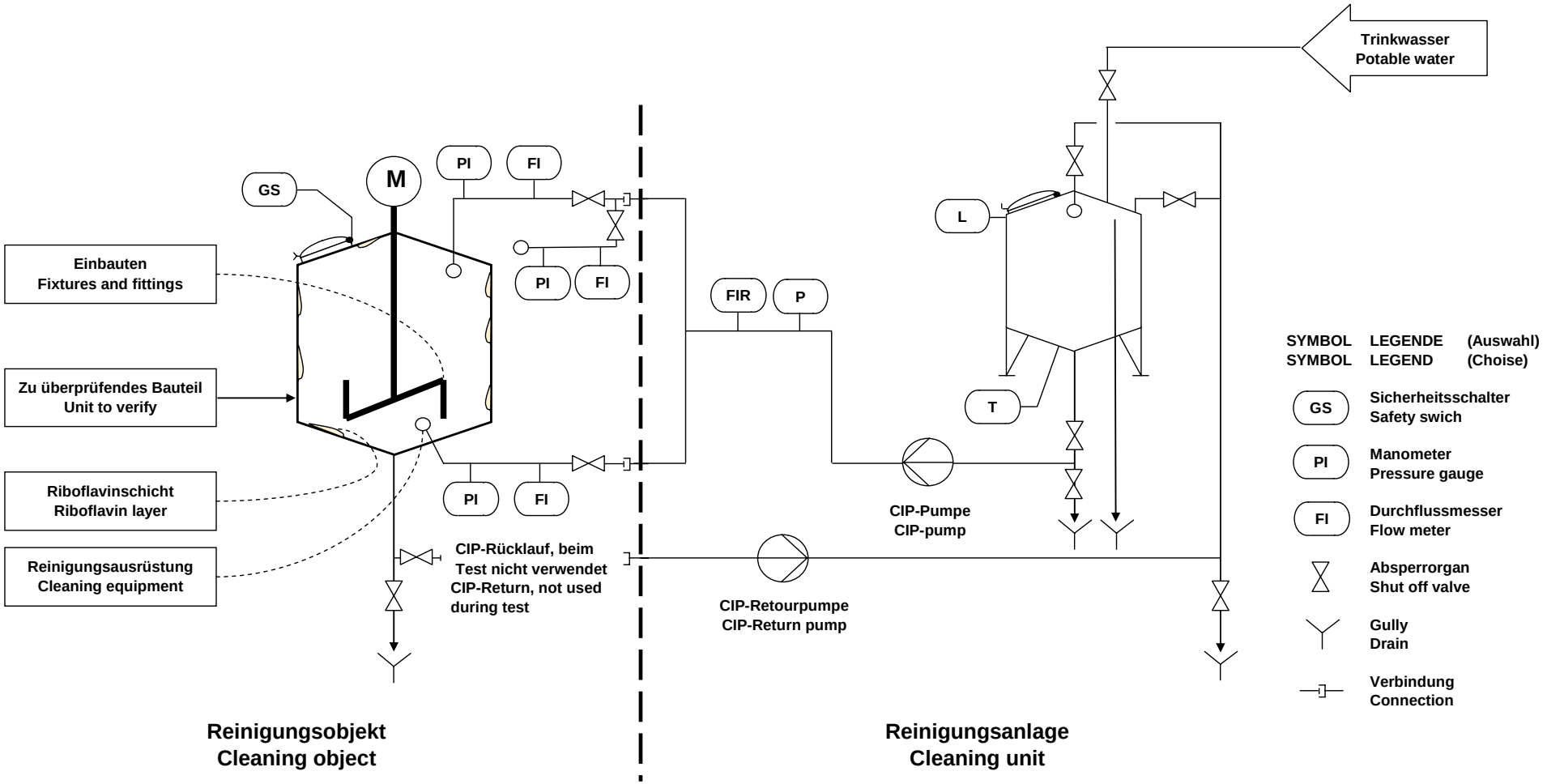

Fig. 1: Schematic sketch of an installation for carrying out a fluorescence test