Swab test of E. Coli cells

Figure 1: TOC-V_{CSH} with ASI-V and SSM-5000A

Additional solid sample module enables swab analysis

Cleaning validation is used to verify the effectiveness of a cleaning procedure for equipment used during drug production. Well-cleaned equipment prevents contamination of the final drug product. Depending on the production and cleaning processes applied, various sampling methods are available to effectively test the cleaning procedure implemented.

In addition to the fast and simple final-rinse sampling method, the swab method for wiping certain areas of the production equipment is becoming increasingly important. The swab test is applied for residues such as coatings, crusts, baked-on residues in corners and edges and substances that are difficult to dissolve. After wiping, the swab material can be extracted in a solvent (with subsequent analysis of the extraction solution) or it can be analyzed directly. This article discusses the direct determination of swabs for testing the presence of E. Coli cells using TOC solid sample analysis.

TOC analysis in cleaning validation

TOC analysis is carried out to determine the sum parameter of the total organic carbon. During TOC analysis, the carbon present in a sample is oxidized to CO_2 and subsequently analyzed using an NDIR detector (non-dispersive IR detector). This ensures fast and simple analysis of aqueous samples (analysis time is approximately 4 minutes).

For this reason, TOC analysis is playing an ever-increasing role in cleaning validation for the determination of the final rinse sample. The TOC value is considered as representative for the level of residual organic contamination of the pre-product as well as the level of cleaning agent residues. In the direct swab method the carbon-free swabs are analyzed directly in the solid-sample module of the TOC analyzer (Fig. 1).

Analysis system and measuring method

The modular design of Shimadzu's TOC-V series supports straightforward combination with a solid sample module (SSM-5000A). In this way, the instruments can also be employed for the analysis of swabs. The SSM-5000A module can be used in combination with TOC-V combustion or wet-chemical models.

For TC determination, the sample (in this case the entire swab) is placed into a ceramic sample boat and is transferred into the combustion oven where it is heated to 900 - 980 °C. At these temperatures, all carbon compounds are oxidized to CO_2. In order to assure complete oxidation, a catalyst is added to the combustion tube.

The CO_2 produced is subsequently transferred to the detector in the main instrument. The NDIR detector of the TOC-V series contains a tandem cell consisting of a long cell and a short cell. The long cell is used for water analysis and the short cell for solid sample analysis.

This configuration assures that the system can be employed for cleaning validation, while retaining its complete flexibility and switching functionality between water and solid sample analysis.

Sample preparation for swab analysis

As TOC analysis results represent sum parameters, it is important to guarantee that the measured total carbon actually originates from the wiped surface. The swab material must therefore be absolutely carbon-free.

In principle, various swab shapes or geometries are possible. This depends on the individual handling and task requirements. Figure 2 shows two examples, a filter and a quartz fiber swab.

The quartz swabs are first heated to approximately 700 °C in a muffle kiln and are moistened with ultrapure water immediately prior to wiping. The quartz fiber swabs are much easier to handle. They do not tear and are easily transferred into the sample cell using tweezers (annealed).

Calibration and measuring results

The SSM calibration is carried out at, in this case, typically low TOC values using a diluted glu-

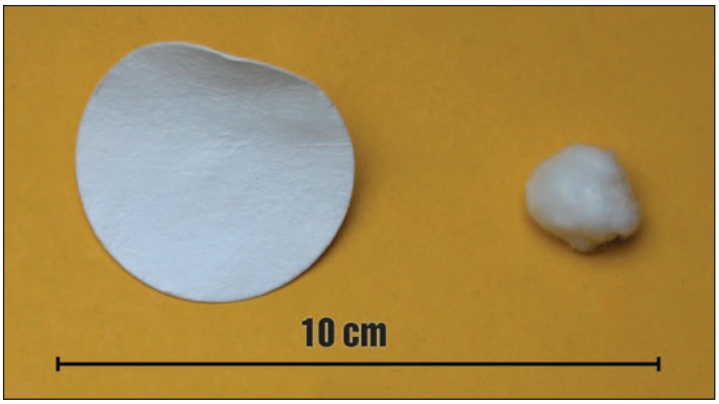

Figure 2: Filter and quartz swab

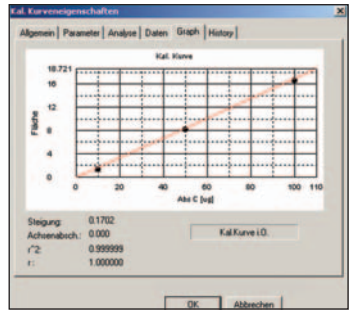

Fig. 3: Calibration curve of 10 - 100 µg carbon

Biopharmaceutical cleaning validation

Glucose solution (0.25 % = 0.1 % µl)	TOC absolute [µg]	Area
10	10	1.25
50	50	8.07
100	100	16.57

Table 1: Calibration values

E. Coli cells [n]	TOC (Swab sample) [µg]	TOC (Control sample) [µg]
Blank value	9.0	0.0
8.00 E+6	15.8	8.2
1.60 E+7	24.8	18.5
3.32 E+7	40.5	29.1

Table 2: Results of the swab and control samples

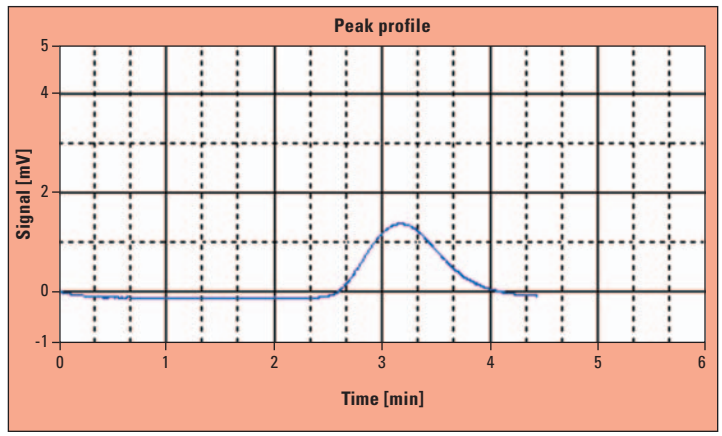

Figure 4: Peak graph of a swab measurement

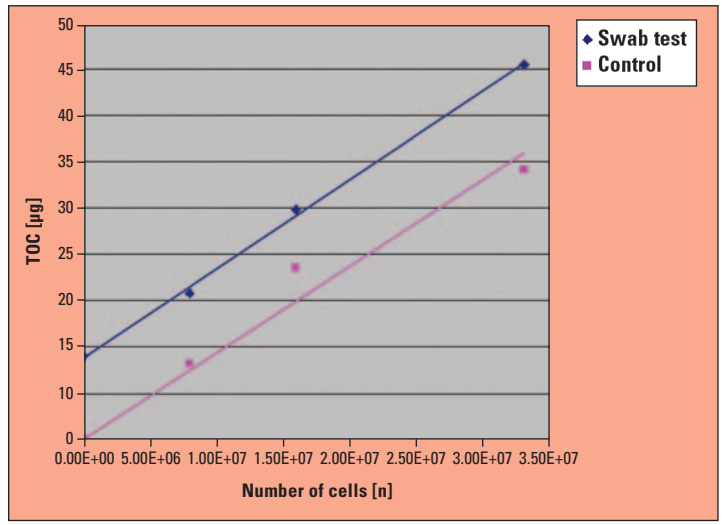

Figure 5: TOC in correlation to the number of E. Coli cells

cose solution for the range of 10 - 100 µg absolute carbon (see Figure 3).

For calibration, only one sample boat filled with ceramic wool was used. For the purpose of cleaning, the boat is heated to 900 °C in the SSM oven. The glucose solution can now be added step by step into the sample boat while remaining in the SSM sample port. The same procedure is applied for the samples. The sample cell remains in the port and the swabs are consecutively transferred into the boat.

Swab method for E. Coli cells

The following example describes the determination of E. Coli contamination of steel Petrie dishes. These serve as testing surfaces for a targeted quantitative contamination with E. Coli cells.

In the present test, three steel Petrie dishes were contaminated with an E. Coli suspension. An additional dish was pre-treated with ultrapure water in order to determine the blank value. The steel surfaces were wiped with annealed (> 30 min at 700 °C) quartz fiber swabs after moistening with 200 µL ultrapure water.

To check the recovery, accurately determined amounts of the E. Coli suspensions as well as blank values (ultrapure water) were applied directly onto the swabs and subsequently measured.

The swabs prepared in this way were subsequently combusted in the SSM-5000A solid sample module at 900 °C in the presence of oxygen and a catalyst. Quantification of the CO_2 combustion product finally enabled TOC (Total Organic Carbon) determination.

Results

The results of the swab samples as well as the control samples are summarized in Table 2.

Figure 5 clearly shows that the contamination can be quantitatively determined using the swab test. The slopes of the straight lines are virtually identical. The parallel shift can be attributed to a blank value arising from the wiping procedure. In terms of arithmetic, this blank value is 9 µg carbon absolute.