Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Preparation Methods of Culture Media for Microbiological Examination of Food

A comparative study on time, energy and water requirements, performed by an independent food QC laboratory

Culture media for microbiological food testing can be prepared in various ways. The traditional method is to dissolve dehydrated media in water and autoclave the media in a suitable vessel. With the help of so-called media preparator instruments, media preparation can be automated to a certain degree. Laboratories can also purchase ready-to-use culture media from suppliers, whereby they avoid having to prepare the media themselves. The methods vary significantly with respect to the work steps, time and resources needed.

A recent innovation in the preparation of microbiological culture media is the ReadyStream® system. At the push of a button, it can prepare up to 100 liters of culture media such as Buffered Peptone Water (BPW) for immediate dispensing when food samples need to be tested.

The ReadyStream® media bags come pre-filled with irradiated and granulated dry culture media (DCM). A 10x concentrated media stock is prepared in the bag by adding water that has been sterile filtered through the ReadyStream® filter set. Whenever media is needed for testing, the right amount of concentrated stock solution, which can be pre-heated, is drawn and diluted with further sterile filtered water. In this way, a total volume of 30 or 100 liters of media per bag can be made available on demand, depending on bag size.

In case it is necessary to dispense the media through a gravimetric dilutor, it is possible to prefill the media into an intermediary bottle, using specific ReadyStream® connector accessories. From this bottle the media can be withdrawn by the dilutor and supplements added.

The aim of this study was to compare the ReadyStream® media preparation workflows with those of commonly used laboratory methods for media production and sample dilution regarding time, energy and water consumption directly at the location where testing is performed. All data in this study were determined by the BAV INSTITUT GmbH, A Tentamus Company, in Germany. The media preparation and dispensing methods that were investigated and compared are:

- ReadyStream® System Direct Dispensing
- ReadyStream® System with Dilutor
- Traditional – Media Kitchen and Autoclave
- Media Preparator Instrument
- Ready-to-Use (RTU) Media Bags

The Life Science business of Merck operates as MilliporeSigma in the U.S. and Canada.

MERCK

Method

- For the measurements, the processes for each of the five methods were divided into sub-activities such as media mixing or cleaning of equipment.
- The total processing time and operator hands-on time were measured. While hands-on time requires active participation from the operator such as weighing the appropriate amount of media, the total processing time encompasses additional activities that do not necessitate direct participation from the operator, for example an autoclave run.
- Energy and water consumption data were taken as published by the equipment manufacturers.
- The activities for warehouse management, incoming goods inspections and performance testing according to ISO 11133 were not considered as these can vary greatly depending on the laboratory's organization. They are usually in the same order of magnitude for all 5 methods.
- The media for the small 25 g food samples were not pre-warmed, whereas those for the large 375 g samples were pre-warmed as required by regulations (EN ISO 6579-1:2017).
- For each of the methods, the reference volume for the comparisons was the production of 100 liters of Buffered Peptone Water (BPW) media.
  - For both ReadyStream® methods, the individual activities for sample preparation were measured 20 times for 25 g samples (each in 225 mL of media) and 10 times for 375 g samples (each in 3375 mL of media). Then the average values were calculated for both sample sizes and these values multiplied by the respective coefficients to determine the values for 100 liters of media.
  - For the Media Preparator Instrument, all measurements were performed for a 20 L media preparator, and the values were extrapolated to a volume of 100 liters of media. However, autoclaving time was factored in only once as autoclaving in several media preparators can take place simultaneously.
  - For the 5-liter ready-to-use media bags, all measurements were performed once and the values multiplied by 20 to determine the values for a volume of 100 liters of media.

Preparation methods and their required activities as determined by process analyses

ReadyStream® System Direct Dispensing

Here, the ReadyStream® System was used in the standard mode as shown in image 1 (without connection to a dilutor).

Activities considered: filter and bag installation, calibration of the water pump (1x every 7 days), media rehydration, calibration of media pump (2x for 100 L media bag), sample preparation and weighing to exactly 25 g or 375 g, media dispensing (flow rate: 2 L/min).

Image 1: The ReadyStream® System, consisting of the media preparation unit with attached filtration unit (left, with filled BPW media bag on top), barcode reader (center) and media dispensing unit (right, with sample and media in stomacher bag)

ReadyStream® System with Dilutor

In this setup, a 5 Liter media bottle was interposed between the ReadyStream® media preparation unit and a dilutor (see **image 2**). The media reservoir bottle can be filled 20 times to deliver 100 L of media of the ReadyStream® System.

Activities considered: filter and bag installation, calibration of the water pump (1x every 7 days), media rehydration, calibration of the media pump (2x for 100 L media bag), connection of the ReadyStream® bag to the reservoir bottle, filling of the 5 L media reservoir bottle, preparation and weighing of the sample, media dispensing via dilutor (flow rate: 0.95 L/min).

Image 2: The ReadyStream® System connected to a dilutor via an intermediary reservoir bottle

Traditional – Media Kitchen and Autoclave

The activities needed to produce 100 L of media directly from dehydrated culture medium were investigated. One hundred 1-liter bottles were filled manually using a measuring cup. Subsequently all bottles were autoclaved in a single autoclave run.

Activities considered: cleaning vessel and bottles, weighing DCM, adding water to boilers, mixing and dissolving the medium, measuring pH, filling into 1-liter bottles manually, loading and starting the autoclave (14.9 kW), testing for sterility and by means of spore strips (1x per autoclave run), preheating bottles in incubator (2 kW; only for 375 g samples), preparing and weighing the sample, adding the medium, pre-rinsing and putting away the bottles, rinse cycle of laboratory sink.

Media Preparator Instrument

Here a 20 L media preparator instrument (4.4 kW) connected to a dilutor was analyzed for the time, energy as well as water needed to produce and process 20 L of media.

Activities considered: cleaning media preparator, weighing DCM, adding water, mixing and dissolving the medium, measuring pH, preparing media preparator, autoclaving, connecting the master clave to the dilutor and calibrating, preparing and weighing the sample, adding the medium via dilutor (flow rate: 0.95 L/min).

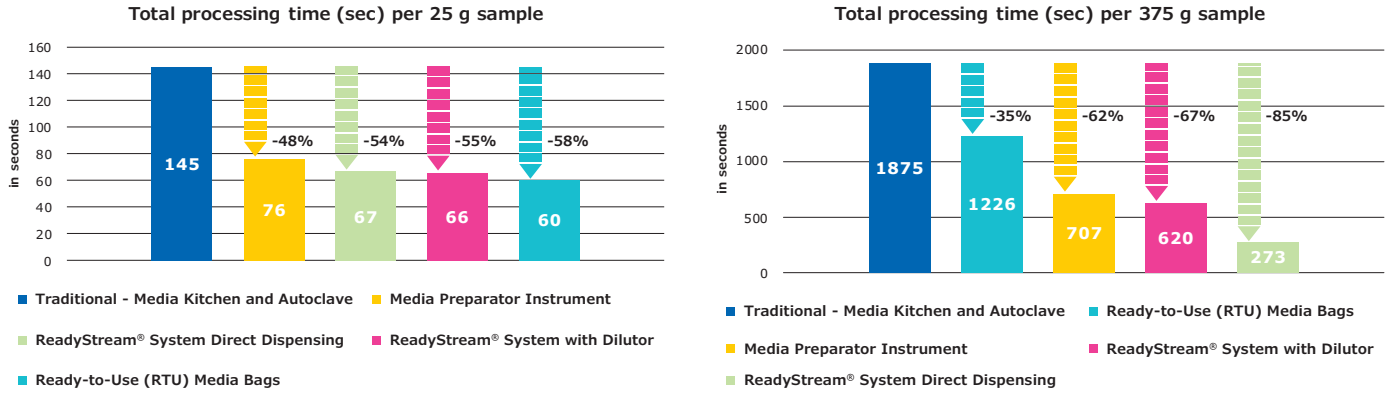
Ready-to-Use (RTU) Media Bags

In this study, the time required to process a 5-liter ready-to-use media bag (catalogue number 0120214-2X5L) for the initial dilution through a dilutor (flow rate: 0.95 L/min) was measured.

Activities considered: preheating the bags in a 2 kW incubator (applies only to 375 g samples), connecting the bag to the dilutor, calibrating, preparing and weighing the sample, adding the medium via dilutor.

Results

The recorded measurements for the total processing times, the operator hands-on times as well as water and energy consumption directly at the laboratory's point of use are illustrated in **figures 1 to 8** below.

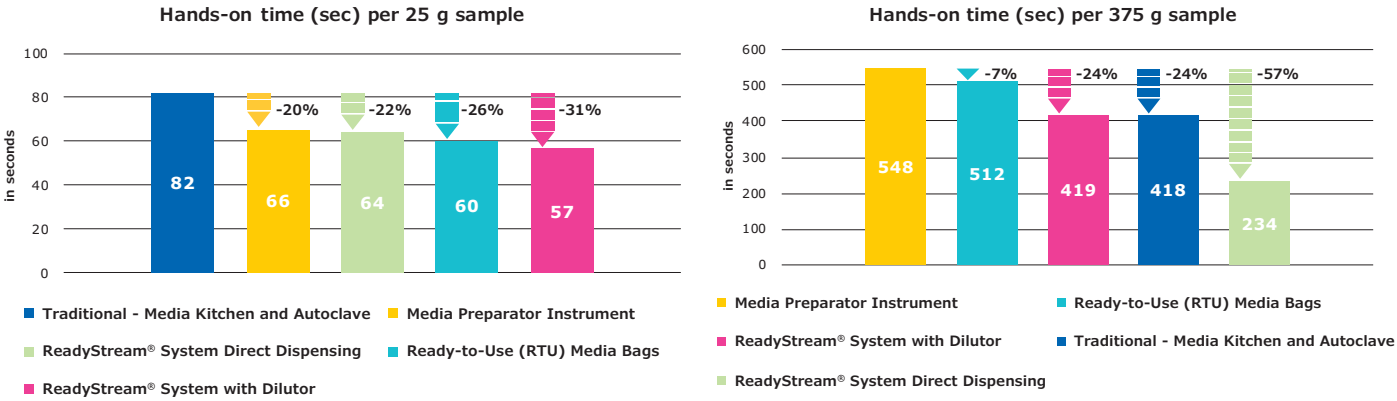


Figures 1 and 2: Illustrations showing, within the columns, the total processing time per 25 g (left) and 375 g (right) sample in seconds for the examined media production methods and, above the columns, the time savings in percent compared with the method that takes the longest.

For small 25 g food samples (**figure 1**), the ready-to-use 5-liter media bags with connection to a dilutor exhibited the shortest processing time, followed by the two ReadyStream® methods. The 5-liter media bag only needs to be connected to the dilutor, whereas the ReadyStream® System requires media production and, when used with a dilutor, filling of the 5 L media reservoir bottle. The time needed for autoclaving but also for cleaning equipment such as the bottles or the media preparator explains the long processing times especially for the *Traditional – Media Kitchen and Autoclave* but also for the *Media Preparator Instrument* method.

The *RTU - Media Bags* require 58% less and the ReadyStream® methods 54% to 55% less processing time than the *Traditional – Media Kitchen and Autoclave* method, which takes the longest of the five considered methods.

For the large 375 g samples (**figure 2**), the ReadyStream® workflows proved the most efficient with regard to processing time. This is mainly due to less or no time needed for various activities that the other methods require for media production. When testing the larger food samples the total processing time of *RTU - Media Bags* increases significantly as one 5 L bag cannot serve to dispense the needed 3.375 L of media for more than one sample. Therefore, bags need to be changed frequently, resulting in more time needed per sample. Furthermore, additional work steps are required for the *RTU - Media Bags* because the larger volumes of media for 375 g samples need to be pre-warmed. Both ReadyStream® methods support automatic pre-warming of the media, so no additional work steps are necessary. The *ReadyStream® System Direct Dispensing* method proved the most efficient for the larger samples, requiring 85% less time than *Traditional – Media Kitchen and Autoclave*.

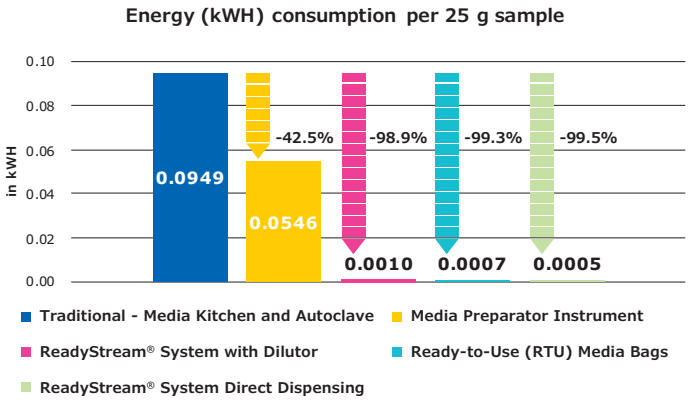


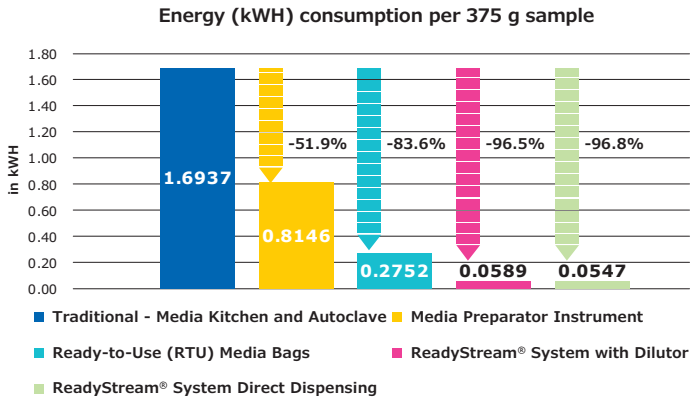
Figures 3 and 4: Illustrations showing, within the columns, the hands-on time per 25 g and 375 g sample in seconds for the examined media production methods and, above the columns, the time savings in percent compared with the method that takes the longest.

Figure 3 shows that for 25 g samples the *Traditional – Media Kitchen and Autoclave* method requires the most operator hands-on time. *ReadyStream® System with Dilutor* proved the most time-efficient of the methods, taking 31 percent less hands-on time than *Traditional – Media Kitchen and Autoclave* and thus saving even more time than with *RTU - Media Bags*. This is mainly because the *ReadyStream® System with Dilutor* method does not need connecting and disconnecting of a new bag after every 5 L of consumption, as the reservoir bottle stays connected to the dilutor.

For samples weighing 375 g (**figure 4**) the hands-on time, like the total processing time (**figure 2**), is by far the shortest with the *ReadyStream® System Direct Dispensing* workflow. An analysis of the individual activities of all the five methods reveals that sample dilution is fastest with the *Traditional – Media Kitchen and Autoclave* workflow as here the media can

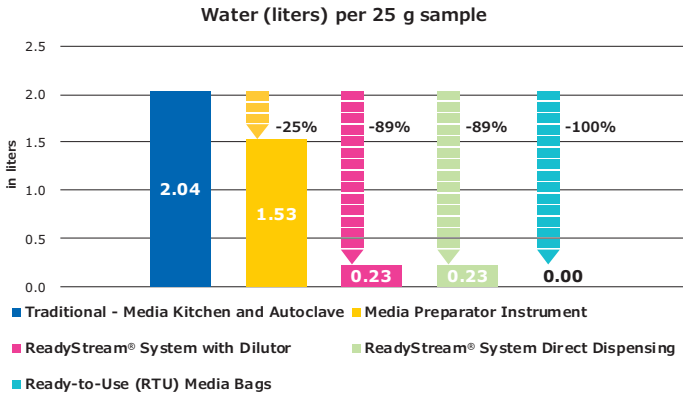
be poured out of the bottle quite quickly. However, when comparing the full workflows with all their activities, including manual production, filling, and autoclaving of the media, the ReadyStream® workflows proved much more efficient.

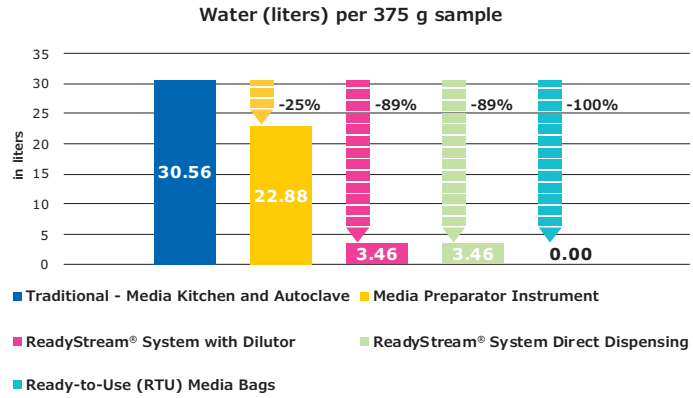




Figures 5 and 6: Illustrations showing, within the columns, the energy consumption per sample in kWh (kilowatt-hour) for the examined media production methods and, above the columns, the energy savings in percent compared with the method that requires the most energy.

For both the 25 g and the 375 g samples, the two ReadyStream® methods and the *RTU - Media Bags* method require much less energy than the conventional methods using DCM to produce the media in the laboratory (**figures 5** and **6**). This is because there is no need for energy-intensive autoclaving of the media and cleaning as well as drying of the equipment used, for example the bottles. The *Traditional – Media Kitchen and Autoclave* workflow also requires pre-warming of the media bottles inside an incubator and thus additional large amounts of energy when testing 375 g samples. This is also the explanation why for the 375 g samples the energy consumption of *RTU - Media Bags* increases in relation to the ReadyStream® methods: the *RTU - Media Bags* need to be pre-warmed in an incubator, too.





Figures 7 and 8: Illustrations showing, within the columns, the water consumption in liters for the examined media production methods and, above the columns, the water savings in percent compared with the method that requires the most water.

With regard to water consumption (**figures 7 and 8**), *RTU - Media Bags* requires no water at the testing location—all the water needed to produce the media is consumed at the supplier's production site. The ReadyStream® workflows are not far behind, requiring only little water consumption, mainly for rehydration of the DCM and for dilution of the 10x concentrated stock solution.

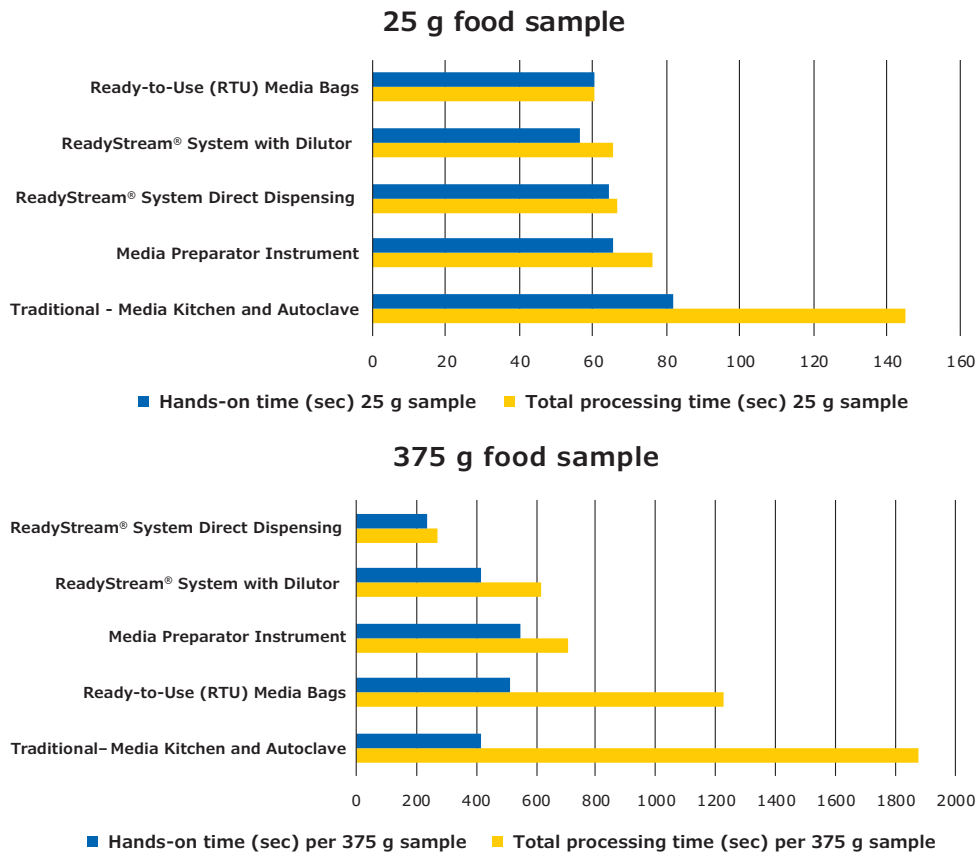
Both the *Traditional – Media Kitchen and Autoclave* and *Media Preparator Instrument* processes consume significantly larger quantities of water than the ReadyStream® workflows due, respectively, to the need to clean the equipment after use and to cool down the instrument.

Conclusion

- With regard to hands-on time, the ReadyStream® methods, and particularly *ReadyStream® System with Dilutor*, proved more efficient than traditional media preparation methods for 25 g samples.
- For the large 375 g samples, the *ReadyStream® System Direct Dispensing* method came out on top for both total processing time and hands-on time.
- The above observations point to the maximum efficiency for both small and large samples.
- Compared to the other methods, both energy and water volumes were lower, resulting in lower costs as well.

Appendix

Figures 9 and **10** summarize the total processing time and hands-on time per 25 g and 375 g food sample for the five examined media production methods.



Figures 9 and 10: IIllustrations showing the total processing time and the hands-on time per 25 g sample (above) and 375 g sample (below) in seconds for the examined media production methods.

To place an order or receive technical assistance

Order/Customer Service: **SigmaAldrich.com/order**
For Technical Service visit: **SigmaAldrich.com/techservice**

SigmaAldrich.com/readystream

We have built a unique collection of life science brands with unrivalled experience in supporting your scientific advancements.

Millipore® Sigma-Aldrich® Supelco® Milli-Q® SAFC® BioReliance®

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

© 2024 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved. Merck, the vibrant M, BioReliance, Millipore, Milli-Q, SAFC, Sigma-Aldrich Supelco, ReadyStream and GranuCult are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

MK_WP13409EN Ver. 1.0
54259
02/2024