Instructions for Use

AMODIA®

easyFlow®
QC Combo 1

Molecular test system
for the detection of

RNA of
Escherichia/Shigella group
Pseudomonas aeruginosa
Staphylococcus aureus

Σ

25

REF:
AEF-QC1

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Content List

1 Components

1.1 Materials Supplied, Storage and Stability

Components	Cat-No.	Content (25 tests)	Preparations	Storage	Stability / Shelf life
Hybridisation					
Suspension Buffer (white cap)	SB03	1 vial a 9 ml	ready to use	4 - 8°C	Until expiration date; 60 days after opening
Hybridization Buffer (blue cap)	HBP03	1 vial a 3 ml	ready to use	4 - 8°C	Until expiration date; 60 days after opening
Probe Mix ES1 (red cap: Escherichia/Shigella)	PMES1	1 vial a 150 µl	ready to use	4 - 8°C	Until expiration date; 60 days after opening
Probe Mix P01 (blue cap: *P. aeruginosa*)	PMP01	1 vial a 150 µl	ready to use	4 - 8°C	Until expiration date; 60 days after opening
Probe Mix S01 (green cap: *S. aureus*)	PMS01	1 vial a 150 µl	ready to use	4 - 8°C	Until expiration date; 60 days after opening
Detection					
Lateral Flow Dipsticks	LFD01	3 vials a 25 Stk.	ready to use	4 - 8°C	Until expiration date; 60 days after opening
Chromatographic Buffer (blue cap)	ChB05	1 vial a 15 ml	ready to use	4 - 8°C	Until expiration date; 60 days after opening

*: LFD vials must be locked tightly! Storage with opened LFD vial reduces the stability of the LFDs.

Important note: Expiration dates should not be exceeded.

1.2 Laboratory Instruments and Materials Required

- Programmed heating/cooling block (ThermoQ)
- Adjustable pipettes for 10 µl, 100 µl and 1.000 µl
- Rack made of plastic or aluminium, suitable for the PCR tubes
- Microwell plate
- evtl. Lysozyme (1 mg/ml, freshly solved in TE buffer 10 mM; pH 8,0)
- Centrifuge for 1,5 ml reaction tubes
- Pipette tips with contamination protection (filter)
- PCR tubes compatible with the heating/cooling block (ThermoQ)
- evtl. Lysostaphine (1 mg/ml, freshly solved in VE water)

1.3 About this Instruction for Use

It is strongly recommended to read and follow the notes and protocols of this instruction for use. Experienced users may use the short protocol.

Technical literature is available at www.amodia.com

For information about changes to the previous version of this instruction for use please contact our technical service.

2 Product Description

2.1 Basic Principle

The easyFlow® Method is based on the detection of ribosomal RNA by a hybridisation with species-specific probes. Theses probes bind to sequences which are characteristic for the respective bacterial species like a fingerprint. As RNA in dead cells degrades quickly, this method detects only viable cells (or "cells alive quite recently"). The easyFlow® test-kits requires no amplification technique (e.g. PCR).

The easyFlow® test-kits consists of the modules RNA extraction, probe hybridisation (to RNA) and detection of the hybridised probe-RNA complex on lateral-flow dipsticks (LFD).

1.) Sample Preparation
The test requires an enrichment culture similar to a bioburden measurement. This ensures that the solution contains enough replicating, i.e. RNA producing, microorganisms for the detection. DNA of non-viable microorganisms is not detected. A centrifugation step concentrates the cells of microorganisms eventually present in the samples and removes residue of the enrichment culture. An optional lysing step may hydrolyse cell walls to enhance RNA availablilty.

2.) Hybridisation
A part of the suspended sample solution is mixed with the probe mix. During the following temperature controlled hybridization reaction the cells are lysed. Labelled probes hybridize to the released ribosomal RNA. This RNA-probe complex is detected directly on the LFD.

3.) Detection
The detection is performed using an immuno-chromatographic assay on a Lateral-Flow Dipstick. With this device the hybridization product is detected.
The hybridized complex of RNA and probes binds to an antibody, which is immobilised on gold particles. With the flow of the chromatographic buffer the complex also flows through the membrane. This membrane is prepared with two lines of different capture molecules. At the first line only gold particles are bound which are also linked to the hybridization product. This binding increases the local concentration of the gold thus forming a visible line. Gold conjugates without hybridization products diffuse further and bind at the second line. Here the line gets visible if the gold conjugate is still intact. This serves as a control for the correct performance of the Lateral-Flow Dipstick.

Lateral-Flow Dipsticks (v. Fig. 1) are used for the detection. They consist of an area of sample application (purple), a membrane and an absorption area (white) With exception of the application area the whole strip is covered by a tape. The strip can be touched at the covered areas. Remarks should be made only on the tape above the absorption area.
Hybridization products are applied directly onto the Lateral-Flow Dipstick.

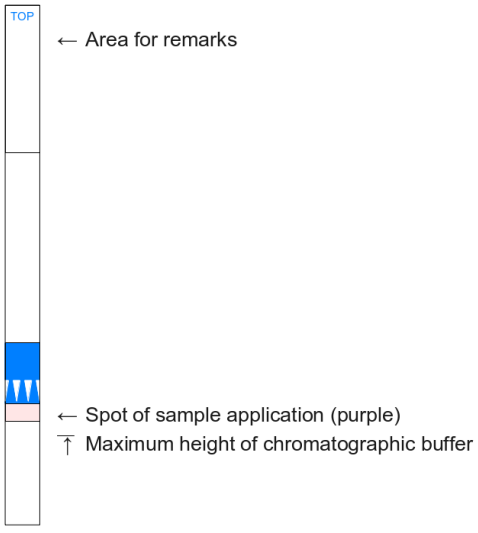


Fig. 1: Layout of the Lateral-Flow Dipsticks

2.2 Kit Specifications

The test-kit easyFlow® QC Combo 1 is intended to be used in microbial quality control. From a fresh enrichment culture three reactions for the species *Pseudomonas aeruginosa* and *Staphylococcus aureus* as well as members of the *Escherichia coli* /Shigella-group are prepared, which directly detect RNA of grown bacteria.

Generating the sample material for this test is typically performed by treating an aliquot of a product sample with an inactivation solution and a subsequent inoculation in an enrichment nutrient. **It is the sole responsibility of the operator to validate that neither the product material, the inactivation solution nor the enrichment solution cause any false results**.

Each of the three specific detecion reactions requires 1ml of the liquid enrichment culture.

Sample material:	Liquid enrichment culture (1ml per reaction)
Analytic specificity:	Probe mix ES1 (Escherichia/Shigella group): Highly specific for bacteria of the Escherichia/Shigella group Probe mix P01 (*P. aeruginosa*): Highly specific for the bacterial species *Pseudomonas aeruginosa* Possible cross-reactions with: *Pseudomonas putida, Escherichia coli* Probe mix S01 (*S. aureus*): Highly specific for the bacterial species *Staphylococcus aureus*

Test time without enrichment culture (using the ThermoQ):

Hybridization set-up	approx. 10 minutes
Hybridization reaction +	approx. 25 minutes
Detection and read-out	approx. 15 minutes
Total test time	approx. 50 minutes

+ : With heating and cooling rates of 1,5°C/s

2.3 Handling, Preparation and Storage of the Materials

2.3.1 Programming the Temperature Profile

ThermoQ	use	lid heating
step 1 (Init)	1 min	at 32°C
step 2 (Denaturation)	5 min	at 95°C
step 3 (Annealing)	5 min	at 50°C
step 4 (Cooling)	1 min	at 28°C

Thermal cycler	Lid heating	110°C
Denaturation	**temperature***	5 min* at 95°C
Annealing	**ramp****	0,1°C/s to 50°C
	temperature*	5 min* at 50°C
Cooling	**temperature***	3 min* at 20°C

*: The specified times given are the reaction times **without** the time required for cooling and heating!

: means: **slow cooling from 95°C to 50°C, followed by a step at **50°C for 5 min**.

The total duration time of the ThermoQ program is approx. 25 min.

2.4 References and Precaution Measures

2.4.1 General References

All reagents of this test-kit are strictly intended for the specified diagnostic use only. The test should only be operated by personnell which has been trained and instructed to perform the test.
Please adhere strictly to the sequence of processing steps provided by this protocol.
Store all reagents in the original vials at the temperatures indicated on the respective labels. Do not interchange kit components of different lots and assays. Do not use kit components beyond their expiration dates.
Stick to the safety rules for handling kit reagents and sample materials. Especially be aware of the following precautions:

- do not eat, drink or smoke
- use pipet tips with contamination protection
- wear safety clothes and gloves
- avoid contact with reagents and sample materials

Some reagents may contain preservation substances against microbial growth, so avoid contact with skin and / or mucous membranes.
Empy vials could be discarded with the normal laboratory waste.

2.4.2 Precaution Measures

Important Notes / Precaution Measures:

- Open and close the reaction vials individually.
- Change gloves immediately, if contaminated.
- Open the reaction tubes carefully in order to avoid aerosols. If possible centrifuge for a few seconds.
- Hybridized products (RNA-probe conjugate) should be treated as an important source of potential contamination.
- Do not interchange components of different lots.

3 Safety Instrutions

The folllowing components of the kit contain hazardous substances:
- None -

Wear gloves and safety glasses. Follow the safety instructions in this chapter.

3.1 GHS Classification

If components of this kit contain substances which are hazardous to health or environment, their concentrations are below 1 percent by weight. No component weighs more than 125g or contains more than 125ml, thus contains no ingredients with miner dangerous properties above these limits. So all components do not have to be labeld with H- und P-codes.

Further information is provided in the safety data sheets (www.amodia.de).

4 Protocols

4.1 Scheme

Fig. 1 shows how to process the three specific detection reactions from one liquid culture. Three aliquots are processed in parallel and hybridized individually with the specific detection reactions, before they are detected on lateral-flow dipsticks.

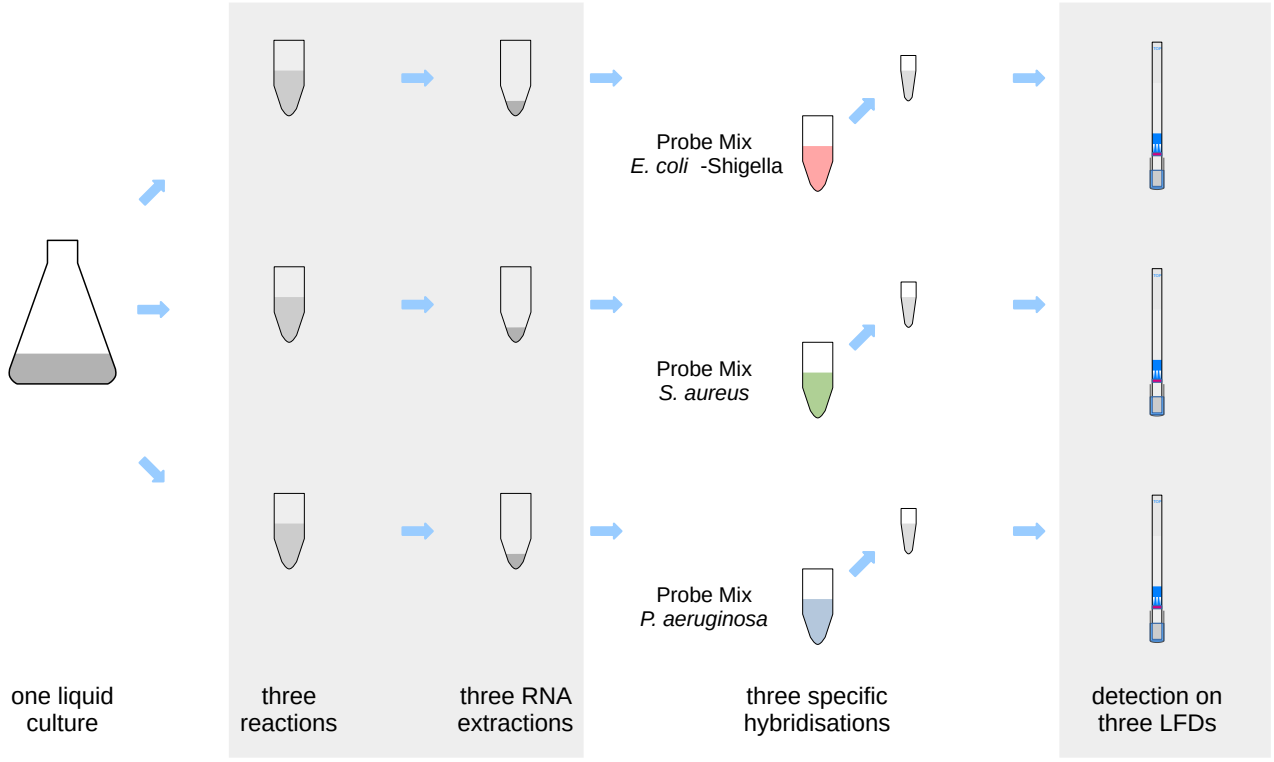


Fig. 2: Scheme to process and detect three different species from one liquid culture

4.2 RNA-Extraktion

1.	Every sample requires **three** sterile 1,5 ml reaction tubes. Label the tubes with the sample ID and **one** letter (E, P, S) for each respective species.
2.	For every reaction transfer **1 ml enrichment culture** into single reaction tubes. For each tube use a new pipette tip. Close the tubes individually.
3.	Centrifuge the samples **at 13´000g for 10 min**.
4.	Remove the supernatant completely from the sediment. For each tube use a new pipette tip. Close the tubes individually. ⚠ Take care to remove **as much medium as possible** from the tube. Otherwise false-positive results are possible.
5.	With a new filter tip for every sample pipet **100 µl suspension buffer** into the reaction tubes. Open tubes **individually** und close them immediately after pipetting.
6.	**Suspend** the sediment in the buffer by **pipetting several times up and down** or **vortex** for **10 s**. The samples are now prepared for the hybridisation reaction.

ℹ **Note**: For gram-positive bacteria (e.g. Staphylococcus sp.) a more sensitive detection may be achieved by using lysing enzymes. The alternative protocol can be found int the chapter "Troubleshooting".

4.3 Hybridisation

4.3.1 Preparation of the Three Master Mixtures

For each species or group a mastermix is prepared. Therefor the volumes for a single reaction have to be multiplied with the factor of reactions (samples + controls + 1).

Hybridisation Mixture		**Example: mastermix for 10 samples and 1 control**
Reagent	**Volume per reaction**	**Factor = 12**
Hybridisation Buffer	**30,0 µl**	360,0 µl
Probe Mix	**5,0 µl**	60,0 µl
		pipet 35 µl into each of the 11 reaction tubes, discard the remaining mixture

After this step **three mastermixes** (one each for the Escherichia/Shigella group, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) with identical volumes should be available.

4.3.2 Steps for Hybridisation

1.	Every sample requires **three** sterile PCR reaction tubes. Label the tubes with the sample ID and **one letter** (E, P, S) for each respective species. Process all pipetting steps without interruption. It is recommended to process a negative control in every test run.
2.	Prepare the three mastermixes (see above).
3.	Pipet **35 µl mastermix** into the **corresponding** reaction tubes (Mix E into tube labeled E, etc.). **Close the reaction tubes.**
4.	Use a new filter tip for every sample to pipet **100 µl sample solution** of 4.2 into the layed-out mixes. Open tubes **individually** and close them immediately.
5.	Negative controls contain no further material.
6.	Place the samples into the prepared ThermoQ (or a thermal cycler) programmed according to 2.3.1. Switch on the ThermoQ (or start the hybridization program on the thermal cycler). Wait for the program to finish.

4.4 Detection on Lateral-Flow Dipsticks

4.4.1 Steps for Detection

1.	Prepare the required number of LFDs and label them, including the letter for the species (E, P, S). Only touch the areas covered with tape and use the white area at the end of the LFD for labeling. Close the LFD vial tightly.
2.	For each reaction pipet **150 µl chromatographic buffer** into wells of a microplate.
3.	Pipet **10 µl** of the hybridisation reaction onto the application area (purple, v. Fig. 1) of the **corresponding** LFD (sample with mix "E" onto LFD "E" etc.). A bleeding of the liquid is normal. Important: Mind the correlation of PCR vials and LFDs!
4.	Dip the LFDs with the area without tape into the chromatographic buffer prepared in step 2. Allow an incubation of **10 min**. Make sure that the application area is discolored. The control line must be visible for interpretation of results. The lines are stable and can be read later.

4.4.2 Interpretation of Results

1.	**Two** lines are visible: test line and control line (v. Fig. 3) **⚠ Attention**: Even a faint test line has to be interpreted as positive. For a comparison the negative control should be considered. If necessary the test must be repeated for confirmation. Positive results may be visible even before the entire incubation time is over.	The detection of RNA of the respective species is **positive.**
2.	**Only one** test line is visible at the location of the control line. **ⓘ Note**: Do not interpret the results until the incubation time is over.	The detection of RNA of the respective species is **negative.**

The result of the test is only valid if the control line of every sample is visible.

The **negative control** for the complete test run has to be correct in order to validate the results. If the negative control shows a visible test line, the analysis of **all** samples tested in parallel must be repeated.

The dried test strips could be adhered to an evaluation sheet and stored.

The test-kit gives only a qualitative result. The intensity of the stained test line has no direct relation to the number of cells present in a positive sample.

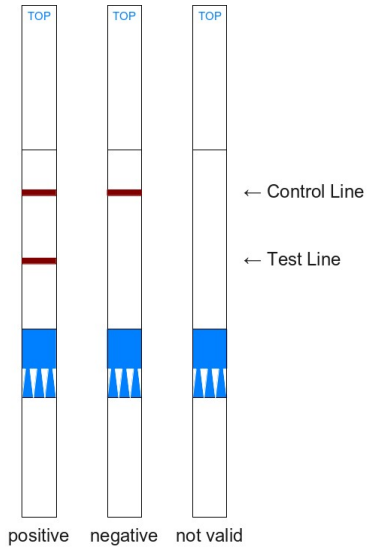


Fig. 3.: Interpretation of results

5 Annex

5.1 Troubleshooting

Problems	Possible cause	Recommendation
No growth in enrichment culture	a) Inappropriate growth temperature	a) Check optimal growth temperature
	b) Inappropriate nutrient solution	b) Use nutrient media (e.g. Caso) instead of selective media
	c) Inactivation solution does not work	c) Use freshly prepared inactivation solution or try different solution
No control line visible (upper line)	a) Chromatographic buffer wrong or disfunctional	a) Use new chromatographic buffer
	b) Test strips expired	b) Use new test strips
	c) Wrong storage of test strips	c) Store sealed at 2 - 8°C
All samples and controls show a positive result.	a) Contamination of hybridization reaction	a) Clean bench and pipettes, check reagents.
	b) Chromatographic buffer is contaminated.	b) Use new chromatographic buffer
Positive control shows a negative signal	a) Wrong hybridization buffer or no probe mix added	a) Repeat hybridization, check reagents
	b) Hybridization failed	b) Check hybridization program
	c) Inhibition by tested product	c) Repeat test, use appropriate inactivation solution or dilution
Weak signals for the detecion of *Staphylococcus aureus*	a) Insufficient cell lysis	a) Use protocol with enzymatic lysis (5.1.1)

5.1.1 Alternative Protocol for RNA Extraction with Enzymes

An alternative to enhance the detrection of gram-positive bacteria (z.B. *Staphylococcus* sp.) may be the folllowing protocol:

1.	Every sample requires **three** sterile 1,5 ml reaction tubes. Label the tubes with the sample ID and **one** letter (E, P, S) for each respective species.
2.	Prepare a solution of Lysostaphine: **1mg Lysostaphine** in **1ml VE water**.
3.	For every reaction transfer **1 ml enrichment culture** into single reaction tubes. For each tube use a new pipette tip. Close the tubes individually.
4.	Centrifuge the samples **at 13´000g for 10 min**.
5.	Remove the supernatant completely from the sediment. For each tube use a new pipette tip. Close the tubes individually. ⚠ Take care to remove **as much medium as possible** from the tube. Otherwise false-positive results are possible.
6.	With a new filter tip for every sample pipet **100 µl Lysostaphine solution** into the reaction tubes. Open tubes **individually** und close them immediately after pipetting.
7.	**Suspend** the sediment in the buffer by **pipetting several times up and down** or **vortex** for **10 s**. Incubate the samples for **10 min at 37 °C**. The samples are now prepared for the hybridisation reaction.

5.2 Ordering Informationen

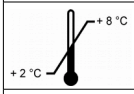
Product	REF	Pack of
AMODIA easyFlow® QC Combo 1	AEF-QC1	25 rx
Suspension Buffer 03	SB03	9 ml
Hybridisation Buffer 03	HBP03	3 ml
Probe Mix ES1 (for Escherichia/Shigella group)	PMES1	150 µl
Probe Mix P01 (for *P. aeruginosa*)	PMP01	150 µl
Probe Mix S01 (for *S. aureus*)	PMS01	150 µl
Lateral Flow Dipsticks 01	LFD01	25 Stk.
Chromatographic Buffer 05	ChB05	15 ml

5.3 Restriction of Use / Warranty

The test-kit easyFlow® QC Combo 1 is designed for microbiological quality control applications. Any use for other applications is in full responsibility of the user and not covered by any warranty.

Several items of the test-kit may interfere with ingredients of users product materials. AMODIA® takes no warranty that the test-kits work with the users product material. **It is the sole responsibility of the operator to validate that neither the product material, the inactivation solution nor the enrichment solution cause any false results**.

5.4 Explanation of Symbols

Symbol	Explanation
REF:	Article Number
	Storage conditions
	Packaging size
	Follow instructions for use

Symbol	Explanation
	Lot identifier
	Expiration date
	Manufacturer
	For evaluation only!

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