Frequently Asked Questions

Malaria-LAMP

EIKEN CHEMICAL CO.,LTD.

Human
Diagnostics Worldwide

Questions & Answers

This document is intended to give a comprehensive overview of important aspects of the Malaria-LAMP method, such as the storage, the handling or equipment characteristics. Only selected information from valid product documents is given. The instructions given in the valid versions of the applicable product documents (IFU, manuals, decontamination regulations) need to be followed when using the respective IVD products.

LAMP Technology

What is the technology behind the Loopamp™ assays?
The LAMP or Loopamp™ technology bases on the amplification of genetic material at one temperature. The generation of a single strand by the DNA helicase, the binding of primer pairs and the generation of copies are optimized to work perfectly at one temperature. LAMP uses 4 primers that detect 6 distinct regions on the target DNA. During the amplification process, copies are generated that contain loop or cauliflower similar structures. That's why the technology is called "loopamp".

What is the difference between LAMP technology and Real-time PCR?

Feature	Real-Time PCR	LAMP technology
Primers per target	2	4
Target numbers	1	6
Amplification	Exponential	Not exponential
Temperature steps for amplification	3	1
Time to result	1–3 h (depending on assay)	50–55 min
Equipment	Thermocycler	HumaLoop T/M or HumaTurb C+A
Results	Qualitative and quantitative	Qualitative
Result reading	Fluorescence curves (software)	Visual (green fluorescence) or real-time turbidity
Sensitivity/Specificitiy	97 - 98%/ 90 - 91%*	96 - 100%/ 97 - 100%

Table 1: Side by side comparison of Real-Time PCR and LAMP technology;
**tested with the artus Malaria RG PCR Kit (Qiagen)*

Is there already an External Quality Assessment for LAMP?
No, unfortunately there is no EQA system yet.

What does the WHO policy brief recommend?
WHO recommends Malaria-LAMP in low-transmission settings for mass screening, Malaria epidemiological surveys and the reactive infection detection after identification of an index case.

What are the advantages in comparison to hypersensitive RDT's?
No, HUMAN offers three different parameters (Pan/Pv/Pf) and the LOD of all tests is 1 parasite/µl. All Malaria-LAMP kits demonstrated also a high specificity by using boil & spin method and PURE.

Reagents (General)

What is the shelf-life of the kits?
Loopamp™ PURE DNA Extraction Kit: 24 months after production
Loopamp™ Malaria Detection Kits: 18 months after production
The usage of the kit after the expiry date is not recommended and off label use (out of warranty).

What are the shipping conditions?
The kits can be shipped at room temperature (2-30°C).
The humidity should be between 0 –80%.

What are the storage conditions of the kits?
The kit should be stored at room temperature (2-30°C). If the room temperature is higher, it is recommended to store the kit in a refrigerator at 2-8°C.

How long does it take to perform a LAMP assay?
The average time from sample to result takes around one 1 h depending on the experience of the user and the sample number. The incubation times for all assays are 5 min for sample lysis using the Loopamp™ PURE DNA Extraction Kit, 40 min amplification and 5 min inactivation. Result interpretation takes around 2 min with the HumaLoop T.

How long does it take to perform different sample numbers (DNA extraction with Loopamp™ PURE DNA Extraction Kit)?

Sample number	Time to result
1 + NC&PC	76 min
3 + NC&PC	77–78 min
7 + NC&PC	83–84 min
10 + NC&PC	87–88 min
14 + NC&PC	95–96 min

Table 2: Time to result with different sample numbers

What is included in the Loopamp™ Detection Kits?
The kits contain reaction tubes with dried reagents, liquid positive and negative controls, 30 µl plastic droppers and the instruction for use in 4 languages (GB, F, E, G).

How do you suggest using the droppers?
Use one dropper for each control. Do not use the same dropper for negative control first and then for transferring the positive control. This might dilute the control and could promote the transfer of contaminations.

Do I get quantitative or qualitative results with the Loopamp™ Detection Kits?
Based upon the LAMP technology, only qualitative results can be obtained.

Which LAMP tests are commercial available?
So far, assays for the detection of MTBC and Malaria Pan, Pf and Pv are available.

If the reagents are stored in the refrigerator, do they have to be equilibrated at room temperature before using them?
Yes, equilibrate them for 15 min at room temperature before start testing.

Why does the Loopamp™ Pure DNA Extraction Kit have 90 extractions but the Loopamp™ MTB Detection Kit has 96 tests?
A maximum of 16 reactions in one run of HumaLoop T can be performed. Of these 16 tests, 14 are samples and two are controls. The negative control will be processed identically as the samples while the positive control is pipetted directly in the amplification tubes. This means : 96 detection tests are needed for six runs of 16 tests, but only 90 Extraction kits are needed, as the positive control is not purified by the extraction kit.

How long are the results stable?
The fluorescence of the results is stable for up to two hours in light surroundings. Then it becomes weaker. They should be interpreted and documented immediately after the run is completed.

Reagents (Loopamp™ PURE DNA Extraction Kit)

How many extractions can be performed with one kit?
The kit contains enough material for 90 extractions.

What does the heating tube contain?
The heating tube contains sodium hydroxide for sample lysis. For a better recognition, the lysis buffer was colored.

Does it have any influence on the test performance, if the heating tube was longer than 5 min in the heating block?
After the 5 min lysis process, the heating block cools down.

How long can I store the dissolved powder-sample mix before the extraction?
It is not recommended to store the resolved powder-sample mix. The extraction should be performed immediately. Otherwise the mix dries quickly.

What is the content of the powder?
The powder contains chelating agents that removes the inhibitors from the lysed sample.

Some power came out when the adsorbent tube was opened. Is it dangerous or harmful?
No.

Is there a processing control for the lysis step?
No.

Can I start the extraction when the powder is not completely dissolved?
No. the power should be dissolved completely before starting the extraction.

A red color appears at the bottom of the adsorbent tube after mixing the powder with the lysed sample. What is the reason?
The reason for the color change is an insufficient mixing process. Mix through roughly until the powder was dissolved completely and the red color vanished.

Why should I perform the negative control with the PURE kit?
The negative control indicates if the lysis buffer, adsorbent tubes and injection cap are free of contaminations and the processing of the workflow was correct.

Can DNA extracted with the Loopamp™ PURE DNA Extraction Kit also be used for other methods?
In theory, the DNA solution extracted from the Loopamp™ PURE DNA Extraction Kit could be used for another PCR test. However, the performance of the Loopamp™ PURE DNA Extraction Kit has never been validated with any other PCR test as an IVD product. Therefore, such PCR tests would need to be tested before use.

Is it possible to store the extracted DNA?
The extracted DNA should be further processed in a timely manner.

Reagents (Loopamp™ Malaria Pan, Malaria Pf, Malaria Pv Detection Kit)

How many tests can be performed with the Loopamp™ Malaria Detection Kit?
The Loopamp™ Malaria Pan Detection Kit contains 96 or 480 tests.
The Loopamp™ Malaria Pf Detection Kit contains 96 tests.
The Loopamp™ Malaria Pv Detection Kit contains 96 tests.

What is included in the Loopamp™ Malaria Detection Kits?
The kits include all reaction tubes with dried reagents, positive and negative controls and droppers.

Is there a minimum sample number for testing for Malaria?
No. Testing can be started with only one patient sample. To increase the cost efficiency, it is recommended to increase the number of patient samples.

Can I use blood from finger pricks for testing?
Yes.

Which Plasmodium species do the assays detect?
Loopamp™ Malaria Pan detects all 4 Plasmodium species (*P. ovale, P. vivax, P. malariae, P. falciparum*).
Loopamp™ Malaria Pf detects only *Plasmodium falciparum*.
Loopamp™ Malaria Pv detects only *Plasmodium vivax*.

Which target sequences do the assays detect?
The target sequence for Loopamp™ Malaria Pan is the mitochondrial DNA of all *Plasmodium* species.
The target sequence for Loopamp™ Malaria Pf is the mitochondrial DNA *of Plasmodium falciparum*.
The target sequence for Loopamp™ Malaria Pv is the mitochondrial DNA of *Plasmodium vivax*.

Which specimens are validated for detecting Malaria Pan, Pf and Pv?
Blood samples collected from finger prick, collected in heparin tubes, whole blood or dried blood spots (6 mm) from whole blood are validated for the kits.

How should dried blood spots be handled?
The wokflow for the dried blood spots is similar to the usual workflow. Just transfer the punched blood spot (6mm) into the heating tube and continue the extraction like with a blood sample.

Which methods do you recommend for the DNA extraction?
Loopamp™ PURE DNA Extraction Kit, boil & spin and the QiaAmp DNA Mini kit are validated for the DNA extraction. If you use the Loopamp™ PURE DNA Extraction Kit, 30µl 334 mM NaCl solution (not included in the Loopamp™ PURE DNA Extraction Kit) should be added to the lysis buffer before adding the sample.

For boil & spin, 60µl extraction buffer (400mM NaCl, 40mM Tris pH 6.5, 0.4% SDS), 1.5 ml tubes, a heating block and a separate centrifuge are needed. For further information, click:

https://www.finddx.org/wp-content/uploads/2016/04/SOP-LAMP-Malaria-Aug2012.pdf

What is the difference in test performance doing the extraction with Loopamp™ PURE DNA Extraction Kit and boil & spin?
The test performances are similar (please refer to Hopkins et al (2013) Marti HP et al. (2016) and Cuadros J et al.).

Which influence does anti Malaria drugs have onto the test performance of Malaria-LAMP?
Atovaquon (1.59 µg/test), Proguanil (20 ng/test), Chloroquin (40 ng/test), Quinin (960 ng/test), Doxycyclinehydrochlorid (360 ng/test), Mefloquin (168 ng/test), Primaquin (18 ng//test) and Artemisinin (93 ng/test) have no influence onto the test performance of the Loopamp™ Malaria Detection Kit (please, refer to the IFU).

Which influence does EDTA have?
EDTA in blood sample can cause false positive samples when the detection procedure with HumaLoop M is used.

Why does EDTA lead to false positive results when Malaria is detected with HumaLoop M?
EDTA interfers with the calcein detection method at lower concentrations than the turbidity detection.

Is it possible to differentiate between other Malaria species than *Plasmodium falciparum* and *Plasmodium vivax*?
No. Only *P. falciparum* and *P. vivax* can be detected separately. Infections with *P. falciparum* are the most severe ones and could be deadly, if they are not treated immediately.

Which advantages does Malaria-LAMP have compared to RDTs?
Malaria-LAMP is a molecular method which is more sensitive and specific than RDTs. Especially in patients with low parasitemia or asymptomatic patients; Malaria-LAMP provides reliable and sensitive results. Therefore, Malaria-LAMP is a helpful tool to detect Malaria in low prevalent countries and to eradicate the disease.

Can Malaria–LAMP be performed with HumaLoop T?
The pre-installed lysis and amplification temperatures of Humaloop T are different from the pre-installed ones in the HumaLoop M. This might lead to an inefficient sample lysis and unspecific amplification as the primers bind specific only at 65°C.

Which other accessories do I need to perform Malaria-LAMP?
A pipette with a filter tip for transferring 30µl blood into the lysis tube and 344 mM NaCl are required if the Loopamp™ PURE DNA Extraction Kit is used. If boil & spin is used, 60µl extraction buffer (400mM NaCl, 40mM Tris pH 6.5, 0.4% SDS), a heating block and a centrifuge are also required.

What are the LOD's of the Loopamp™ Malaria Detection Kits?
The LOD for the detection of Malaria Pan is 5 copies/test.
The LOD for the detection of Malaria Pf is 7.5 copies/test.
The LOD for the detection of Malaria Pv is 7.5 copies/test.

How many parasites can the assays detect in 1 ml patient blood?
The assays detect 1000 parasites in 1 ml blood. That means 1 parasite in 1µl blood.

When should I test for Malaria?
Early and accurate diagnosis of malaria is essential for both effective disease management and malaria surveillance. High-quality Malaria diagnosis is important in all settings as misdiagnosis can result in significant morbidity and mortality. The symptoms of a Malaria infection are unspecific and similar to flu-like symptoms. Therefore, it is recommended to test for Malaria in high prevalent countries and in low prevalent countries, if the patient has travelled to high prevalent countries (for further recommendations, please refer to http://www.who.int/malaria/areas/diagnosis/overview/en/).

Why is the positive control not handled exactly like the negative control to verify that the extraction was performed correctly?
The positive control is only designed for performance control of the reagent to avoid incorrect results due to quality degradation. NC is only purified water to control contamination in the test environment.

Is it possible to detect malaria infections with HR2 mutations with Malaria-LAMP?
Since Malaria-LAMP recognizes the target gene (the mitochondrial DNA) the HR2 variation (i.e. the encoding sequence for HR2 protein in genome DNA) doesn't affect the detection and performance of Malaria-LAMP.

Instruments (General)

What is the warranty time of the instruments?
One year with option to extend it up to three years.

How often should the instruments be cleaned and maintained?
Once a year, depending on how often the instrument is used and how clean the environment is.

What is needed for maintain the instruments?
The required tools and materials are listed in each Maintenance checklist of the respective instrument. Detailed information will be given during the 'train the trainer' Training. Basically the engineer should be at least in possession of
- measuring temperature unit
- temperature measuring set
- Keypad/controller
- cover for heating chamber (HumaLoop)

What is the life expectancy of the instruments?
The life expectancy of all instruments is five years.

Do the instruments need a UPS?
A UPS should be used especially in areas where problems with sustained electricity supply appears. HUMAN provides UPS for 220 V countries and for HumaLoop T/M and HumaTurb. For all other countries, a UPS must be procured that meets all characteristics listed on the specification flyers.

Which time period can the UPS bridge?
The total duration of the 800VA UPS is 60-65 minutes, depending on the breaks between the different steps. The UPS should only be used for power failures up to one hour. The UPS does not serve to bridge an entire run.

Is it possible to operate the instruments with batteries or solar panel?
Yes, HUMAN provides also a solution with a solar panel and battery

If the temperature exceeds the recommended tolerance of +/- 1 degree Celsius, will it have any impact on the LAMP reaction?
If the temperature of the reaction is out of range, the risk and actual rate of false-negative and false-positive results increases. Please control temperature properly.

If the measured temperature exceeds +/- 3 degrees Celsius, should the engineer still proceed to calibrate the instrument? If they proceed to calibrate the instrument, will "emoticons" be displayed?
If you can't adjust the temperature within the controllable range of +/- 3°C, please replace the instrument.

HumaLoop T/M or HumaTurb C+A need to be send back. What is the disinfectant procedure?

Precautions of LAMP related instruments

If you are returning the LAMP instrument from the customer's laboratory, you should thoroughly clean it using the following procedure to prevent infection or contamination (in the absence of customer instructions).

- **Materials required**
  1. Disposable lab coats ☐
  2. Disposable plastic gloves ☐
  3. Masks (N95 etc.) ☐
  4. 0.5% Sodium hypochloride ☐
  5. 70% EtOH ☐
  6. Paper towels (Kimwipes etc.) ☐
  7. Vinyl bags (for packing, bin bag etc.) ☐

Dispose of all used reagents, laboratory coats, gloves, masks, laboratory articles, etc. in accordance with local regulations!

- **Points to be checked before operation (confirm the experiment situation from the customers)**
  1. Has there been any problem with data or device during their experiment?

     ☐ No

     ☐ Yes What happened? _____

  2. Did they use DNA derived from clinical samples?

     ☐ No

     ☐ Yes What kind of samples did they use? _____

  3. Has a cap from a reaction tube been removed or have amplification products leaked from the reaction tube etc. after DNA amplification?

     ☐ No

     ☐ Yes How many times? _____

- **Decontamination procedure**
  1. Wear a disposable lab coat, a mask and gloves before operation. ☐
  2. Test the negative control included in the kit (N=3) and then confirm whether a DNA contamination occurs or not.* ☐
  3. Prepare the 0.5% Sodium hypochloride solution. ☐
  4. Confirm that no reaction tube etc. remains in the device. ☐
  5. Apply the solution from step 3 onto a paper towel (e.g. Kimwipes) and wipe the device with it. Then leave it at room temperature for 5 minutes. ☐
     → Refer to the separate sheet about cleaning the device.
  6. After 5 minutes, wipe the instrument with a paper towel soaked in 70% EtOH. ☐
  7. Test the negative control included in the kit (N=3) and make sure whether the DNA contamination has been removed or not.** ☐
  8. Seal the device with the vinyl bag and take it to a place where the packaging can be done (outside the laboratory). ☐
  9. Pack the sealed device into the storage box. ☐

**This step can be skipped if there is little possibility of contamination (from the customer information). In this case, however, you should confirm whether a contamination is detected at step 7 or not.*
***According to the situation, you can work elsewhere (e.g. your office) than in the customer's laboratory.*

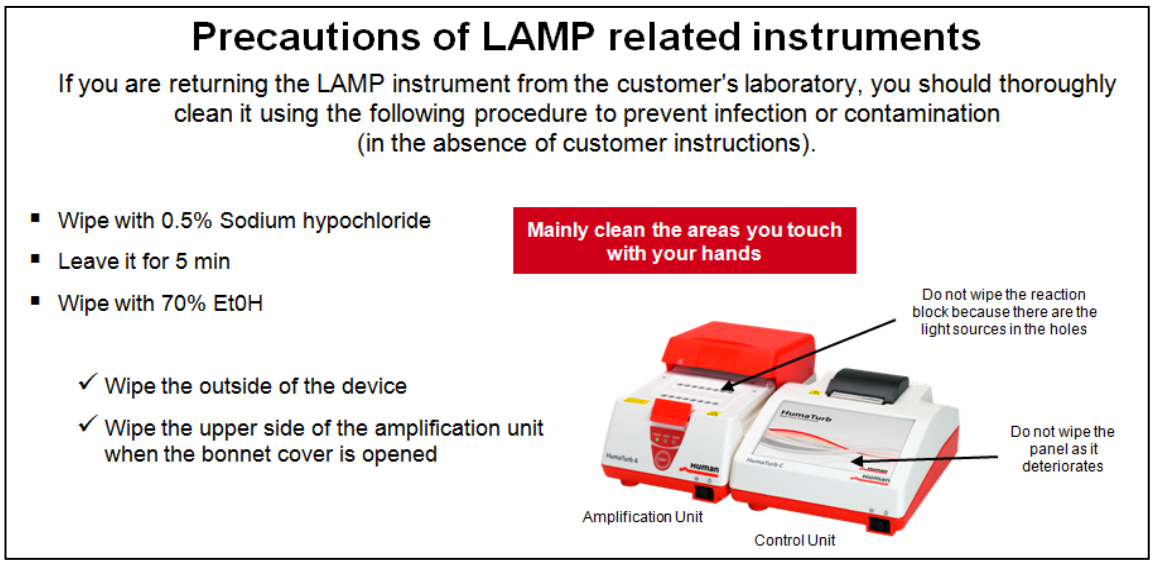

Figure 1: Checklist by Eiken Chemical Co., Ltd.

How to interpret results which are false LAMP negative?

The most common reason for false negative results with LAMP is an inhibition to LAMP reaction from the specimen.

How long can the HumaLoop T/M and the HumaTurb C+A be operated with HUMAN's Solar Panel & Battery solution?

The performance of the battery is sufficient for about two complete runs with the LAMP instruments. The battery can also be charged directly with the solar panel and the time be extended. To charge a fully discharged battery with the solar panel, about 4-5 hours of sunlight are required when using a 100W solar panel.

Is it possible to connect a PC to the instruments?

No, unfortunately it is not possible to connect a PC. Results generated with the HumaTurb C+A system can be transferred to the PC using a USB stick.

Instruments (HumaLoop M)

What is the principle behind the detection of pathogens by reading green fluorescence?
The reagents of the Loopamp™ Detection Kits contain the fluorescent dye calcein which is initially combined with manganese ions to achieve the quenching effect. During the amplification of target DNA, pyrophosphate ions are generated that bind and remove manganese ions from calcein to irradiate fluorescence. The fluorescence is further intensified as calcein combines with magnesium ions. With a UV light source in the fluorescence detection unit, the fluorescence can be detected visually.

What is sample capacity of HumaLoop M?
With HumaLoop M, a maximum of 16 tests can be performed at the same time. For the amplification reaction, up to 14 patient samples can be tested. One positive and one negative control should be performed with each run.

What is the difference between the HumaLoop T and the HumaLoop M ?
Both instruments contain pre-installed heating profiles (incubation temperatures and times) for sample processing and amplification. The pre-programmed profiles support a failsafe operation of the instruments and the tests. HumaLoop T profile is dedicated to the detection of MTBC while HumaLoop M is dedicated to Malaria Pan, Pv and Pf (the instrument might be used in future for other parameters). Therefore, HumaLoop T should not be used for the detection of Malaria and HumaLoop M should not be used for the detection of tuberculosis.

Can the test results be printed out?
HumaLoop M does not contain a printer. The results should be documented separately in a written format.

What is recommended for cleaning HumaLoop M?
In order to ensure the normal operation, detection and use, the instrument needs to be cleaned regularly depending on the lab conditions and usage of the instrument. For cleaning outer surfaces, use a soft and dry cloth to wipe the shell of the device. In case of dirt that cannot easily be removed, use cloth soaked with a small amount of neutral detergent diluted with water. In case of a contamination with template DNA, wipe the instrument with 0.5% hypochlorite solution.

Instruments (HumaTurb System)

What is the HumaTurb system?
The HumaTurb system consits of an amplification unit (HumaTurb A) and a control unit (HumaTurb C). Up to six separate HumaTurb A units can be connected to one HumaTurb C. Each HumaTurb A unit contains two independ reaction blocks with a capacity of 2x8 tests. That means, two different parameters can be performed in one HumaTurb A unit simultaneously. For sample processing with the Loopamp™ PURE DNA Extraction kit, a separate heater with a specific insert for the heating tubes is required (HumaHeat; REF 964000). All available Loopamp™ Detection Kits can be performed with the HumaTurb system at the same time. The result reading is different from HumaLoop - turbidity is measured in real-time during the amplification process.

What is the principle of the real-time detection of turbidity with the HumaTurb system?
During the amplification process, in positive samples, magnesium pyrophosphate is generated that causes turbidity of the reaction mix.

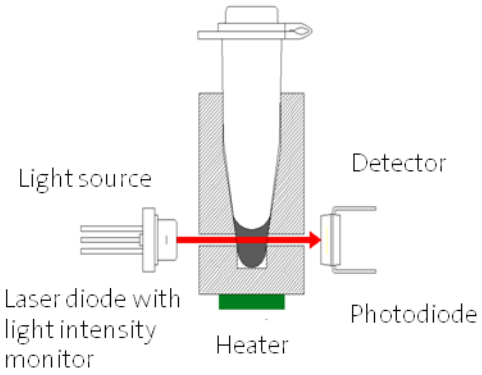

Figure 3: Principle of the real-time turbidimeter HumaTurb C+A.

Under each cavity, a photometer detects the specific absorbance in real-time which is displayed in the measurement screen of HumaTurb C. The interpretation of results is performed in correlation to the absorbances obtained from negative and positive controls and shown in colors (judgment card).

Card color	Judgment result
Pink	Positive (larger than the judgment value)
Green	Negative (lower than the judgment value)

Table 5: Judgment card for result interpetation with the HumaTurb system

Does the HumaTurb system provide quantitative results?
The HumaTurb system provides qualitative results.

What is the sample capacity of the HumaTurb system?
Depending on how many HumaTurb A units (up to six) are connected to HumaTurb C, the test capacity varies from up to 16 to 96 per run. One positive and one negative control should be performed for each assay and each unit.

Can the test result be printed out or transferred?
Yes, the HumaTurb system has a printer, USB and LIS connectivity for transferring the test results.

What is the difference between the test performance of Malaria-LAMP with HumaLoop M and HumaTurb C+A?
Both instruments are using the same kits. The test performance of these two instruments is comparable.

Instruments (HuMax ITA)

What is the advantage of using HuMax ITA?
HuMax ITA automates the 2 min incubation time and all mixing and spinning down steps during the generation of the reaction mix and reduces the manual steps during the LAMP workflow.

Do I have to use HuMax ITA?
No. All published data are generated without using HuMax ITA.

Instruments (HumaHeat)

What is the (sample) capacity of the HumaHeat?
The HumaHeat has a capacity for 16 heating tubes.

Which kind of tubes can I use for the sample lysis with the HumaHeat?
The inserts of the HumaHeat are specially adopted to the heating tubes of the Loopamp™ PURE DNA Extraction Kit.

Can I perform the sample lysis for Malaria and TB with the HumaHeat?
Yes, the incubation temperature can be set manually or with the software.

Advantages

- Usability -> Easy result interpretation
- Robustness -> Accurate pipetting is not mandatory
- Test Performance -> Comparable to other molecular methods
- Flexibility -> Adaptable to all sample throughputs
- Easy logistics -> Due to room temperature shipment & storage (2...30°C)

Notes

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