Holidic media (HM) preparation

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Abstract

This protocol is part of the manuscript: Gonçalves et al. Commensal bacteria and essential amino acids control food choice behavior and reproduction. Plos Biology. 2017 Apr 18.

Holidic media (HM) were developed in collaboration with the laboratories of Linda Partridge at UCL and Matthew Piper at Monash University.

The publications describing the holidic medium development are:

Piper MDW, Blanc E, Leitão-Gonçalves R, Yang M, He X, Linford NJ, et al. A holidic medium for Drosophila melanogaster. Nat Methods. 2013;11: 100–105. doi:10.1038/nmeth.2731

Piper MD, Soultoukis GA, Blanc E, Mesaros A, Herbert SL, Juridic P, et al. Matching Dietary Amino Acid Balance to the In Silico Translated Exome Optimizes Growth and Reproduction without Cost to Lifespan. Cell Metab. 2017;25: 610-621. doi:10.1016/j.cmet.2017.02.005

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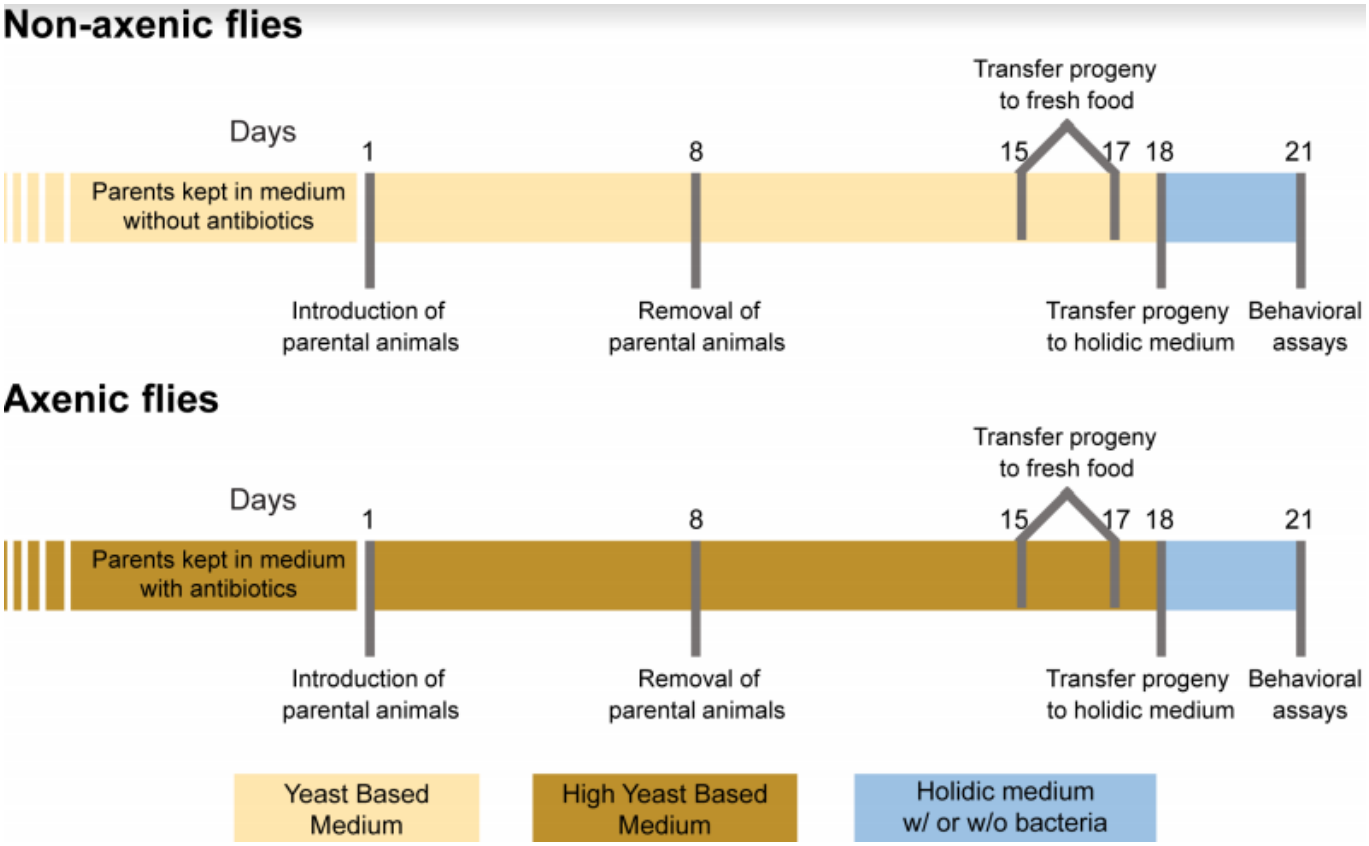
Guidelines



In this publication, holidic media (HM) were prepared as described previously [1] using the HUNTaa formulation, with the exception of the HM used for pretreating axenic and gnotobiotic flies, for which we used an HM with an improved AA composition (FLYAA) [2]. In all experiments where we refer to all neAAs removal, L-Glutamate was still present in the diet in order to prevent any possible adverse effects in neuronal function.

Stock solutions for HUNTaa and FLYAA holidic media preparation

1. Acetate buffer (10X)

- to ~500 ml water, add 30 ml of acetic acid and 30 g KH_2PO_4, then slowly add 10 g $NaHCO_3$ as this will froth as CO_2 is released
- fill up with milliQ water to just under 1000 ml
- the final pH should be 4 otherwise use either HCl or NaOH to adjust to pH 4
- fill up with milliQ water to exactly 1000ml
- filter sterilize and store at 4ºC

2. Cholesterol (66.67X)

- weigh 1 g of cholesterol and fill up to 50 ml using absolute Ethanol
- stir the mix for 15 min until everything is dissolved
- aliquot into 15 ml Falcons and store at 4ºC
- before use, warm to 40ºC for 30 min and vortex to re-dissolve the cholesterol

3. Trace solutions ($CaCl_2.6H_2O$, $MgSO_4$, $CuSO_4.5H_2O$, $FeSO_4.7H_2O$, $MnCl_2.4H_2O$, $Zn\ SO_4.7H_2O$) (1000X)

- add 1000 ml of water to 250 g $CaCl_2.6H_2O$
- add 1000 ml of water to 250 g $MgSO_4$
- add 1000 ml of water to 2.5 g $CuSO_4.5H_2O$
- add 1000 ml of water to 25 g $FeSO_4.7H_2O$. To avoid problems with $FeSO_4$ rusting and dropping out of solution, aliquot and store at -20ºC. To use thaw aliquot once.
- add 1000 ml of water to 1 g $MnCl_2.4H_2O$
- add 1000 ml of water to 25 g $Zn\ SO_4.7H_2O$
- filter sterilize each solution except for $FeSO_4$, aliquot into 2 ml Eppendorf tubes and store at -20ºC
- thaw every aliquot only once, the solution keeps for up to a week when stored at 4ºC

4. Nucleic acids and lipid-related metabolites (125X)

- weigh 6.25 g choline chloride, 0.63 g myo-inositol, 8.13 g inosine and 7.5 g uridine and fill up to 1000 ml using milliQ water
- filter sterilize, aliquot into 50 ml Falcons and store at 4ºC wrapped in aluminium foil to minimize light exposure

5. Vitamins (71.5X)

- weigh 0.100 g thiamine (aneurin), 0.050 g riboflavin, 0.600 g nicotinic acid, 0.775 g Ca pantothenate, 0.125 g pyridoxine and 0.010 g biotin and fill up to 1000 ml using milliQ water
- filter sterilize, aliquot into 15 ml Falcons and store at -20ºC
- thaw every aliquot only once, the solution keeps for up to a week when stored at 4ºC wrapped in aluminium foil to minimize light exposure

6. Folic acid (1000X)

- to dissolve 0.5 g of folic acid in 1000 ml of milliQ water add NaOH (2M) dropwise
- filter sterilize, aliquot into 2 ml Eppendorf tubes and store at -20ºC
- thaw every aliquot only once, the solution keeps for up to a week when stored at 4ºC wrapped in aluminium foil to minimize light exposure

7. Essential amino acids HUNTaa diet (10X)

- dissolve 5.2 g phenylalanine, 4 g histidine, 7.6 g lysine, 3.2 g methionine, 3.2 g arginine, 8 g threonine, 11.2 g valine and 2 g tryptophan into 350 ml milliQ filtered water and vigorously stir until it is dissolved. The solution is close to saturation and therefore requires at least 2 hours to dissolve and may need to be warmed up to 65-75ºC while stirring vigorously. Isoleucine and leucine are handled separately as their solubility is very low.
- wash the sides of the flask with a plastic pipette to ensure everything goes into solution.
- adjust pH to 4.5 using 5 M HCl and fill up to 400 ml with milliQ filtered water
- filter sterilize and store at 4ºC wrapped in aluminium foil to minimize light exposure, the solution keeps for up to two months.

8. Non essential amino acids HUNTaa diet (10X)

- dissolve 14 g alanine, 0.2 g cysteine, 6.8 g aspartic acid, 12.8 g glycine, 6.8 g asparagine, 6 g proline, 10 g glutamine and 7.6 g serine into 350 ml milliQ filtered water and vigorously stir until it is dissolved. The solution is close to saturation and therefore requires at least 2 hours to dissolve and may need to be warmed up to 65-75ºC while stirring vigorously. Tyrosine is handled separately as its solubility is very low. Glutamic acid is added separately for flexibility - if an amino acid or a group of amino acids are omitted, the amount of glutamic acid can be adjusted to compensate for the loss of nitrogen.
- wash the sides of the flask with a plastic pipette to ensure everything goes into solution.
- adjust pH to 4.5 using 1 M HCl and fill up to 400 ml with milliQ filtered water
- filter sterilize and store at 4ºC wrapped in aluminium foil to minimize light exposure, the solution keeps for up to two months.

9. Essential amino acids FLYAA diet (10X)

- dissolve 6.66 g phenylalanine, 4.32 g histidine, 9.02 g lysine, 3.98 g methionine, 10.78 g arginine, 7.32 g threonine, 7.94 g of valine and 2.12 g of tryptophan into 350 ml milliQ filtered water and vigorously stir until it is dissolved. The solution is close to saturation and therefore requires at least 2 hours to dissolve and may need to be warmed up to 65-75ºC while stirring vigorously. Isoleucine and leucine are handled separately as their solubility is very low.
- wash the sides of the flask with a plastic pipette to ensure everything goes into solution.
- adjust pH to 4.5 using 5 M HCl and fill up to 400 ml with milliQ filtered water
- filter sterilize and store at 4ºC wrapped in aluminium foil to minimize light exposure, the solution keeps for up to two months.

10. Non essential amino acids FLYAA diet (10X)

- dissolve 7.28 g alanine, 7.74 g aspartic acid, 5.08 g glycine, 6.8 g asparagine, 6.46 g proline, 7.42 g glutamine and 9.12 g serine into 350 ml milliQ filtered water and vigorously stir until it is dissolved. The solution is close to saturation and therefore requires at least 2 hours to dissolve and may need to be warmed up to 65-75ºC while stirring vigorously. Tyrosine is handled separately as its solubility is very low. Glutamic acid is added separately for flexibility - if an amino acid or a group of amino acids are omitted, the amount of glutamic acid can be adjusted to compensate for the loss of nitrogen.
- wash the sides of the flask with a plastic pipette to ensure everything goes into solution.
- adjust pH to 4.5 using 1 M HCl and fill up to 400 ml with milliQ filtered water
- filter sterilize and store at 4ºC wrapped in aluminium foil to minimize light exposure, the solution keeps for up to two months.

11. Glutamic acid for HUNTaa and FLYAA diets (100 mg/ml)

- dissolve 20 g of glutamic acid in 200 ml of milliQ filtered water and stir until it is dissolved. If using the free acid, glutamic acid will only go into solution when NaOH is added dropwise.
- filter sterilize and store at 4ºC wrapped in aluminium foil to minimize light exposure, the solution keeps for up to two months.

12. Cysteine for FLYAA diet (50 mg/ml)

- dissolve 10 g of cysteine in 200 ml of milliQ filtered water and stir until it is dissolved.
- filter sterilize and store at 4ºC wrapped in aluminium foil to minimize light exposure, the solution keeps for up to two months.

13. Nipagin (100g/l)

- dissolve 10 g of nipagin in 100 ml of milliQ filtered water and stir until it is dissolved.
- store at room temperature in aluminium foil to minimize light exposure.

References

1. Piper MDW, Blanc E, Leitão-Gonçalves R, Yang M, He X, Linford NJ, et al. A holidic medium for Drosophila melanogaster. Nat Methods. 2013;11: 100–105. doi:10.1038/nmeth.2731

2. Piper MD, Soultoukis GA, Blanc E, Mesaros A, Herbert SL, Juridic P, et al. Matching Dietary Amino

Acid Balance to the In Silico Translated Exome Optimizes Growth and Reproduction without Cost to Lifespan. Cell Metab. 2017;25: 610-621. doi:10.1016/j.cmet.2017.02.005

Before start

Make sure all solutions are available in enough quantities.

Protocol

Step 1.

Make the calculations according to the amount of food you need to prepare, considering that we use 6.5 ml of medium per each fly culture vial. To account for pipetting errors it is advisable to do the calculations for the total amount of medium assuming a volume of 7.5 ml of medium per vial. You may use the accompanying Excel files to calculate the amount of reagents required for the desired final volume and nutritional content (HUNTaa or FLYAA).

First part of the medium preparation

Step 2.

Thaw the trace solutions and put cholesterol into the 40ºC water bath for approximately 10 to 15 minutes.

Mix very well by vortexing before using the solutions.

DURATION
00:10:00

First part of the medium preparation

Step 3.

In the following steps we describe how to prepare 1 l of different holidic media, as example. Mark a Schott flask with a pen at the level of 680 ml using milliQ filtered water before adding the different components. Remove the water.

First part of the medium preparation

Step 4.

NOTES
Carlos Ribeiro 22 Mar 2017

Most solid components will only go into solution after being autoclaved

Autoclaving or storing the first part of the medium

Step 5.

Autoclave the solutions right away (121ºC for 20 min) or leave overnight at 4ºC.

DURATION
00:40:00

ANNOTATIONS
Lenny Teytelman 28 Apr 2017

The timer indicates 40 minutes, but the step description says to autoclave for 20 minutes. Is the extra 20 minutes for cooling?

Second part of the medium preparation

Step 6.

While the first part of the medium is being autoclaved thaw vitamins and folic acid solution at room temperature. Make sure to do so avoiding light exposure.

Second part of the medium preparation

Step 7.

While the first part of the medium is being autoclaved prepare the second part of the medium. Mark a Schott flask with a pen at the level of 320 ml using milliQ filtered water before adding the different components. Remove the water.

Second part of the medium preparation

Step 8.

Depending on the nutrient content of the medium add all the following components. At the end, if required, add milliQ filtered water until the pen mark.

Referred to as	holidic medium (HUNTaa)	-all Aas (HUNTaa)	-eAAs (HUNTaa)	-neAAs (HUNTaa)	-single eAA (HUNTaa)	-neAAs + 1xTyr (or 2xTyr) (HUNTaa)	-neAAs + 1xPro (HUNTaa)
Essential amino acids	60.51 ml	0 ml	0 ml	140.45 ml(a)	140.45 ml(a,c)	140.45 ml(a)	140.45 ml(a)
Non-essential amino acids	60.51 ml	0 ml	97.52 ml(a)	0 ml	0 ml	0 ml	0.27 g proline(f)
L-Glutamate	15.13 ml	0 ml	24.38 ml(a)	35.11 ml(a)	35.11 ml(a)	35.11 ml(a)	35.11 ml(a)
Nucleic acids & Lipids	8 ml	8 ml	8 ml	8 ml	8 ml	8 ml	8 ml
Vitamins	14 ml	14 ml	14 ml	14 ml	14 ml	14 ml	14 ml
Folic acid	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Acetic acid buffer	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

Referred to as	-single eAA (h) (HUNTaa)	-folic acid (HUNTaa)	-metals (HUNTaa)	-nc.ac. & lipids (HUNTaa)	-sterol (HUNTaa)	-vitamins (HUNTaa)	holidic medium (FLYAA)
Essential amino acids	60.51 ml(c)	60.51 ml	60.51 ml	60.51 ml	60.51 ml	60.51 ml	60.51 ml(j)
Non-essential amino acids	60.51 ml	60.51 ml	60.51 ml	60.51 ml	60.51 ml	60.51 ml	60.51 ml
L-Glutamate	15.13 ml	15.13 ml	15.13 ml	15.13 ml	15.13 ml	15.13 ml	15.19 ml
L-Cysteine (HCl)	n/a	n/a	n/a	n/a	n/a	n/a	6.83 ml
Nucleic acids & Lipids	8 ml	8 ml	8 ml	0 ml	8 ml	8 ml	8 ml
Vitamins	14 ml	14 ml	14 ml	14 ml	14 ml	0 ml	21 ml
Folic acid	1 ml	0 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Acetic acid buffer	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml

(a) The amount of these nutrients was increased to adjust concentration of biological active nitrogen to 197,9 mM as in the complete HM. In diets where neAAs were removed, L-Glutamate was still added.
(c) A complete essential AAs solution or an essential AA drop-out solution lacking L-Arg, L-Met, L-Val, L-Phe, L-His, L-Lys, L-Thr or L-Trp were used.
(f) The amount of Proline used corresponds to the biological active nitrogen equivalent to 0,42 g of L-Tyr (2,32 mmol).

(j) A complete essential AAs solution or an essential AA drop-out solution lacking L-His were used.

Combining both solutions

Step 9.

When the first part of the medium has cooled down enough (you should be able to touch the sides of bottle, roughly 60 °C), add the second part of the medium to the first part of the medium and swirl vigorously.

NOTES

Carlos Ribeiro 11 Apr 2017

If you are using preservatives, add the corresponding volumes of nipagin and propionic acid at this stage.

Step 10.

Add 6.5 ml of medium to each vial using a multipipetter while still warm and before it solidifies.

Step 11.

Let the medium solidify for 1 hour before adding flies. Protect the box with the vials without lid from contamination by covering it with a bouffant hair protection cap.

DURATION

01:00:00

Step 12.

To ensure optimal access of the flies to the nutrients, the flies should be transfered to new medium every 3 days.