FN3PT, National Federation of Seed Potato Growers,
43-45 rue de Naples, 75008 Paris, France

INRA, National Institute for Agronomic Research
UMR BiO3P1099, 35653 Le Rheu Cedex

Crystal violet pectate single layer medium preparation

SL-CVP$_{AG366}$

(From **V. Hélias**, P. Hamon, E. Huchet, J.V.D. Wolf, and D. Andrivon, 2011. Two new effective semi-selective crystal violet pectate (CVP) media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathology*, 61, 339-345; *valerie.helias@rennes.inra.fr*)

Crystal violet mix	**In 500 ml distilled water :**
▪ $CaCl_2.2H_2O$	1.02 g
▪ Tryptone	1.0 g
▪ Tri-sodium citrate	5.0 g
▪ Na NO3	2.0 g
▪ Crystal violet (0.1%)*	1.5 ml
▪ Agar	4.0 g

Pectin mix	**In 500 ml distilled water :**
▪ NaOH (5M)	2.8 ml
▪ Pectin Dipecta (ref AG366)	18.0 g

- Introduce ingredients of both mixes in the order of the component list.
- Dissolve each ingredient of the crystal violet mix by stirring the medium before adding the following one.
- Stir the pectin mix and heat it up (80-100°C) to allow the pectin to be dissolved avoiding formation of lumps.
- Sterilise both mixes at 120°C for 15 min
- Pour slowly the crystal violet mix into the pectin mix while still hot, by gently (to avoid bubble formation) stirring the medium (using a magnetic stirrer).
- Verify the pH being between 6.8-7.4
- Distribute in Petri dishes the resulting medium immediately after autoclaving.
- Allow them drying to eliminate condensation in a laminar flow cabinet
- Store the CVP$_{2005}$ medium at 4°C.
- Petri dishes are dried with the lids ajar in the laminar flow cabinet to eliminate the condensation before use.

* Please note that this amount is a correction to the original publication, which incorrectly described the use of 1.0% crystal violet for the formulation of SL-CVP.

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Crystal violet pectate double layer medium preparation

DL-CVP$_{AG366}$

(From **V. Hélias**, P. Hamon, E. Huchet, J.V.D. Wolf, and D. Andrivon, 2011. Two new effective semi-selective crystal violet pectate (CVP) media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathology*, 61, 339-345; *valerie.helias@rennes.inra.fr*)

Basal layer medium

$CaCl_2$, $2H_2O$	5.5 g
Tryptone	1 g
Crystal violet 0,1%	1.5 ml
NaNO3	1.6 g
Agar	15 g
Distilled water	1000 ml

- Introduce ingredients in the order of the component list.
- Dissolve each component by stirring the medium before adding the following one.
- Sterilise the basal layer medium at 120°C for 15 min
- Pour the medium in Petri dishes (**15ml** / dishes) in a laminar flow cabinet
- Allow them to set before pouring the overlayer medium

Overlayer medium

EDTA 5,5%,(pH 8.0)	2 ml
NaOH 5M	4.8 ml
Distilled water	800 ml
Pectin Dipecta (ref AG366)	20 g

- Heat up (80-100°C) and stir the overlayer mix to allow the pectin to be dissolved
- Distribute the medium in 2 X 400 ml bottles before autoclaving (120°C for 15 min).
- Verify the pH (6.8-7.4) before pouring
- Pour the medium (**7 ml**) when the basal layer is dry
- Petri dishes are dried in a laminar flow cabinet for 24 to 48 hours
- The DL-CVP$_{AG366}$ medium is stored at 4°C until used
- Petri dishes are dried with the lids ajar in the laminar flow cabinet to eliminate the condensation before use.

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Liquid Enrichment Medium **LEM$_{AG366}$** preparation

(From **V. Hélias**, P. Hamon, E. Huchet, J.V.D. Wolf, and D. Andrivon, 2011. Two new effective semi-selective crystal violet pectate (CVP) media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathology*, 61, 339-345; *valerie.helias@rennes.inra.fr*)

Liquid Medium composition

▪ NaOH 5N	20 µl
▪ $MgSO_4$, $7H_2O$	0.0375 g
▪ $(NH)_4SO_4$	0.1 g
▪ K_2HPO4	0.1 g
▪ Pectin Dipecta (ref AG366)	0.17 g
▪ H2O	qsp 100 ml

- Mix the NaOH solution in water. Add the component in the following order : $MgSO_4$, $(NH)_2SO_4$ and K_2HPO_4 and finally the pectin
- Sterilise both mixes at 120°C for 15 min
- Verify that the pH is comprised between 6.8 et 7.4
- Store the LEM$_{AG366}$ at 4°C.
- Enrichment procedure is applied by adding 10 volumes of LEM$_{AG366}$ to 1 volume of sample to be tested

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