


Simplified Oocyte Vitrification Protocol for HSV Device



For MII Oocytes – Gradual Exposure to ES

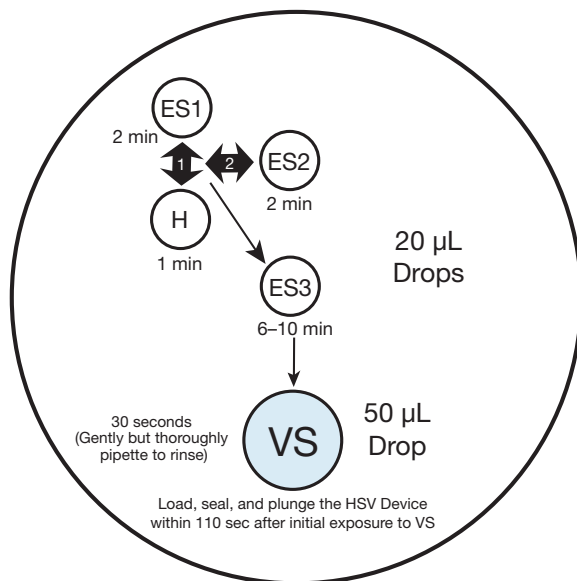
ALL PROCEDURES MUST BE PERFORMED AT ROOM TEMPERATURE (22–27°C)



 As referenced in clinical literature, we recommend oocyte vitrification to be performed within 2 hours from time of oocyte retrieval.¹

Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure.

- Aseptically dispense:
 - one (1) 20 μ L drop of H
 - three (3) 20 μ L drops of ES (ES1, ES2, and ES3)
 -  ES1 and ES2 should be in close proximity to H (but not touching).
- Place MII oocyte(s) (2 maximum), into H and expose undisturbed for 1 minute.
- Merge ES1 with H. Allow spontaneous mixing for 2 minutes.

Use tip of transfer pipette to move ES1 towards H until drops merge.
- Then merge ES2 with H+ES1. Allow spontaneous mixing for 2 minutes.
- Transfer oocyte(s) from merged drop to ES3, and expose undisturbed for 6–10 minutes.
- During the 6–10 minute exposure, aseptically dispense one (1) 50 μ L drop of VS.
- Transfer oocyte(s) from ES3 to VS for 30 seconds before loading.
- Gently but thoroughly pipette oocyte(s) once within VS drop to ensure complete rinse in VS.
 -  To minimize floating, after 10 seconds pipette the specimen(s) to the bottom center of the VS drop.
- Load, seal, and plunge HSV Device (per device instructions) within 80 seconds, not to exceed 110 seconds after initial exposure to VS.
- Refer to HSV Device Loading Protocol, FIS1 P/N 002120 diagram and product insert for detailed loading instructions.
 -  See reverse side for tips.



KEY	
H	HEPES buffered medium with protein (eg., mHTF- HEPES with 20% SSS or equivalent)
ES	Equilibration Solution
VS	Vitrification Solution
	Merge drops
	Transfer specimen to next drop

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Tips

- All procedures are to be done at ROOM TEMPERATURE (22–27°C).

Do not use heated stage.

- Have all necessary materials, tools, and equipment ready and easily accessible before starting procedure.
- HSV Device should be pre-labeled with patient information and the thin end of the blue plastic insertion device should be connected to the colored end of the handling rod.
- Where possible, select only the best quality MII oocytes for vitrification.
- The recommended HSV Device capacity is a MAXIMUM of 2 specimens.

- Process only as many specimen(s) as will be loaded per HSV Device at one time.
- Minimize exposure of specimens to light during equilibration in ES and VS solutions.
- Transfer specimens between drops using a minimal volume of medium.
- The timing for exposure to VS is CRITICAL:
 - Maintain microscopic visualization of specimen(s) by adjusting focus as needed during rapid exposure to VS (specimens will float in the drop).
 - Keep transfer pipette tip close to drop for quick manipulations.
 - Load, seal, and plunge the HSV Device within 80 seconds, not to exceed 110 seconds after initial exposure to VS.

1. Song WY, Sun YP, Jin HX, et al. *Chin J Obstet Gyn.* 2010; 45(8):578-82.

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