

2000 — Pioneering Automated Islet Isolation & Clinical Grade Purification in the LUMC

Michel P.M. van der Burg †



*Automated isolation, IsletPrep, and Visipaque-ViaSpan:
the road to the LUMC's Clinical Islet Laboratory...*



Automated isolation • IsletPrep • Visipaque-ViaSpan : the road to the LUMC's Clinical Islet Laboratory...

- The LUMC Clinical Islet Transplant project's 2000 series of human islet isolations marked the transition from manual to automated pancreatic digestion, using the Ricordi method.
- This shift prompted the drafting of our core SOPs and the training of the required isolation team.
- Islets purified with "IsletPrep" — a reformulated Iodixanol-UWS solution developed with Nycomed/Axis-Shield — showed outcomes equivalent to the 1999 manual-method series: high purity, no islet loss, and preserved integrity.

...

Automated isolation • IsletPrep • Visipaque-ViaSpan : the road to the LUMC's Clinical Islet Laboratory

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- As GMP production of IsletPrep would not be ready in time for the planned start of work in the GMP Facility, we validated a simpler alternative combining clinical-grade Visipaque and ViaSpan, which matched IsletPrep's results in both human and porcine islet purification.
- This Visipaque-ViaSpan protocol became the standard, enabling the opening of the LUMC's Clinical Islet Laboratory (Klinisch EilandLaboratorium) in April 2001.

Human islet purification is hampered by many donor and isolation factors ...

- that may lead to :
 - ...deterioration of the density difference between islets and the pancreatic acinar cells ,
 - ...or the isolation of islets still covered with a mantle of acinar cells — “mantle islets” —
- which generally results in a poor purity and islet loss after density separation .

1997— Purification in Iodixanol - UWS

- We developed in 1997 a highly efficient density gradient of Iodixanol (Optiprep*) in the University of Wisconsin solution (UWS) in the difficult pig model in our islet research laboratory in the Leiden University Medical Center (LUMC).

Van der Burg MPM, Basir I, Bouwman E. No porcine islet loss during density gradient purification in a novel iodixanol in University of Wisconsin solution. Sixth Congress of the Int. Pancreas and Islet Transplant Association (IPITA), Milan (Italy) Sept. 24–27, 1997.

In : Innovative Islet Purification in Iodixanol-UWS • IPITA 97 • Michel van der Burg • Miracles.Media • @1MEMO 20250128 • URL michelvanderburg.com/2025/01/28/

*OptiPrep is a 60% iodixanol solution produced by Axis-Shield, formerly Nycomed (Oslo, Norway)

1998 — Purification in Iodixanol - UWS

- Next, in 1998, our first pilot with Iodixanol-UWS for human islet purification was performed during islet isolation with the automated method at the Diabetes Research Institute in Miami , confirming the high efficacy of this innovative purification solution, with > 80% purity and recovery of the human islets.

Van der Burg MPM, Ranuncoli A, Molano R, Kirlew T, Ringers J, Bouwman E, Ricordi C. Efficacy of the novel iodixanol-UWS density gradient for human islet purification (Abstract). Acta Diabetol 1998; 35: 247

In : Efficacy of the novel iodixanol-UWS density gradient for human islet purification • Igls Aidspit 1999 • Michel van der Burg • Miracles.Media • 20241202 • URL michelvanderburg.com/2024/12/02/

1999 — Purification in Iodixanol - UWS

- In 1999, the LUMC clinical islet transplant project was launched with a first series of human islet isolations using the manual isolation method followed by Iodixanol-UWS purification.
- Parallel efforts with Nycomed* aimed to commercialize our Iodixanol-UWS purification solution , as a ready-made product, coined “IsletPrep”.

1999 — Pioneering the LUMC Clinical Islet Transplant Project • 1MEMO_20260705 • Michel van der Burg • Miracles•Media • URL michelvanderburg.com/2026/07/05/

Islet purification from bench to bedside • @1MEMO_20260623 • Michel van der Burg • Miracles.Media • URL michelvanderburg.com/2026/06/23/

*February 2000 Nycomed Diagnostics was acquired by Axis-Shield

2000 — Automated Islet Isolation & IsletPrep-UWS

- The 2000 series reported here , introduced the 'automated method' for islet isolation , and IsletPrep-UWS purification , to facilitate...

...the upcoming GMP production of islets in our islet laboratory in the GMP Facility of the LUMC

...pre-commercial testing of IsletPrep* for the planned commercial production of our Iodixanol-UWS purification solution as a ready-made cGMP certified product

*IsletPrep production planned by Axis-Shield (formerly Nycomed) : as a 2-component kit with bottles of Iodixanol (Optiprep) and double-strength UWS (UWS2x)

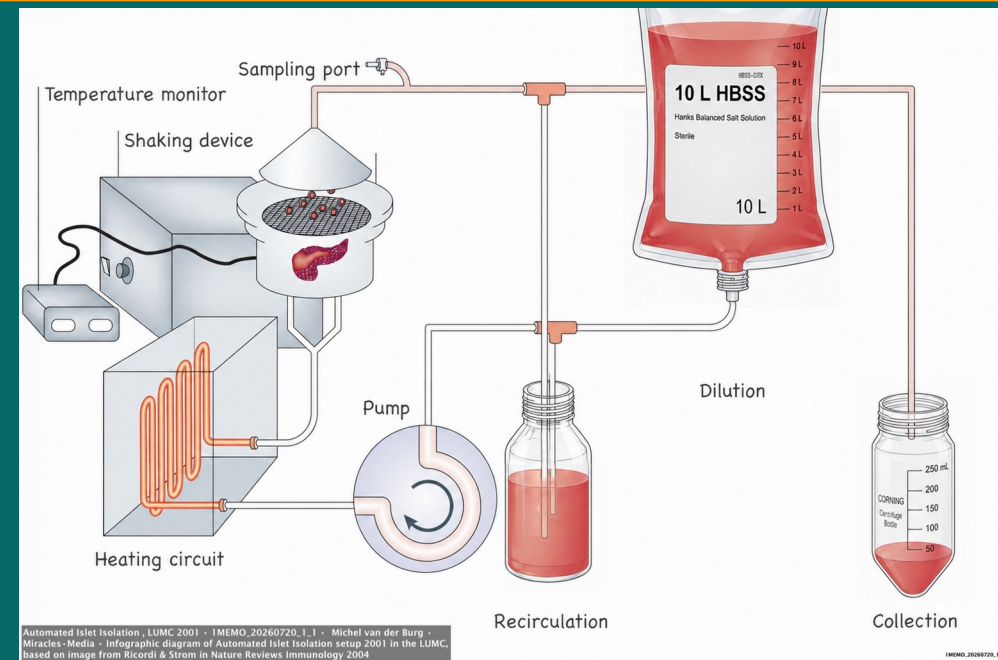
Islet Isolation : Automated Method

- Donor pancreases*
 - distantly procured from multiorgan (15–68 y) donors, were
 - cold preserved with UWS for 4-18 h
- Pancreas digestion
 - by the automated method with collagenase (Liberase-HI) in Hanks
 - digest was collected with room-temperature Hanks, and
 - incubated in cold UWS for 60 min on ice, prior to IsletPrep purification

*Marginal donor pancreases rejected for transplantation

Automated method of the digestion of the pancreas (recirculation and dilution)

- The pancreas is in a steel chamber with glass beads ('knikkers')
- A screen in the chamber allows only particles < 0.5 mm to pass
- The collagenase solution is pumped through a heating coil — warmed to 37°C — and recirculated through the chamber
- The pancreas is progressively broken down into small pieces and after ~ 30 min when most islets are free the recirculation and heating is stopped
- Next, ~ 30 min a room-temperature Hanks solution (HBSS) is pumped into the chamber, and the digest leaving the chamber is collected



IsletPrep Production

Pre-Commercial Manufacturing UWS2x

- IsletPrep production is planned in collaboration with Axis-Shield (formerly Nycomed) as a 2-component kit with bottles of Iodixanol (Optiprep) and double-strength UWS (UWS2x)
- This new IsletPrep edition of the Iodixanol-UWS purification solution uses a slightly modified double-strength UWS composition (UWS2x).
- For the pre-commercial tests in this series of islet isolations in 2000, the IsletPrep was manufactured in-house at the LUMC.

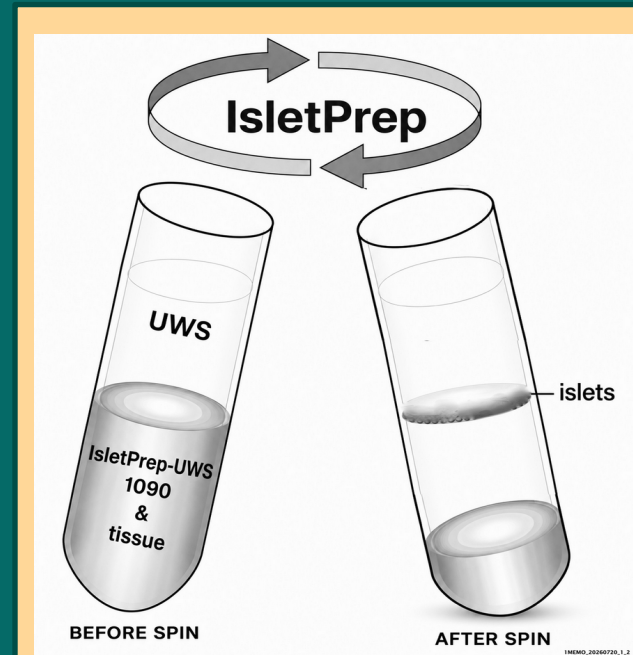
UWS₂x for IsletPrep

Component • Step	MW	mM	Source	Gram / L UWS ₂ x
Aqua Dest (start volume for 1 L UWS ₂ x)			ca 690 ml	
Lactobionic acid	358	100	Sigma Aldrich 15351-6	71.7
NaOH pellets	40	25	Sigma S 8045	2.0
KH ₂ PO ₄	136	25	Sigma P 0662	6.80
KOH pellets	56		Sigma P 5958	11
MgSO ₄	120	5	Sigma M2643	1.20
Raffinose	594	30	Sigma Aldrich 20667-9	35.6
Hydroxyethylstarch	200000	0.25	HES 200, Serum-Werk	100
Make up to 1 liter & adjust pH 7.4, using KOH pellets (ca 1 gram)				0.9 gram

IsletPrep-UWS

Density Gradient Composition

- IsletPrep is prepared by mixing equal volumes of :
 - IsletPrep Part-1 (UWS_{2x}) , and
 - IsletPrep Part-2 (60% iodixanol; OptiPrep)
- A density gradient bottom is prepared by mixing the pancreatic digest tissue (in UWS) with IsletPrep
- E.g. an 1090 bottom is prepared by mixing :
 - 200 ml digest (in UWS) , and
 - 78 ml IsletPrep
- Likewise , a 1090 test tube bottom , by mixing
 - 5 ml digest (in UWS) , and
 - 1.95 ml IsletPrep



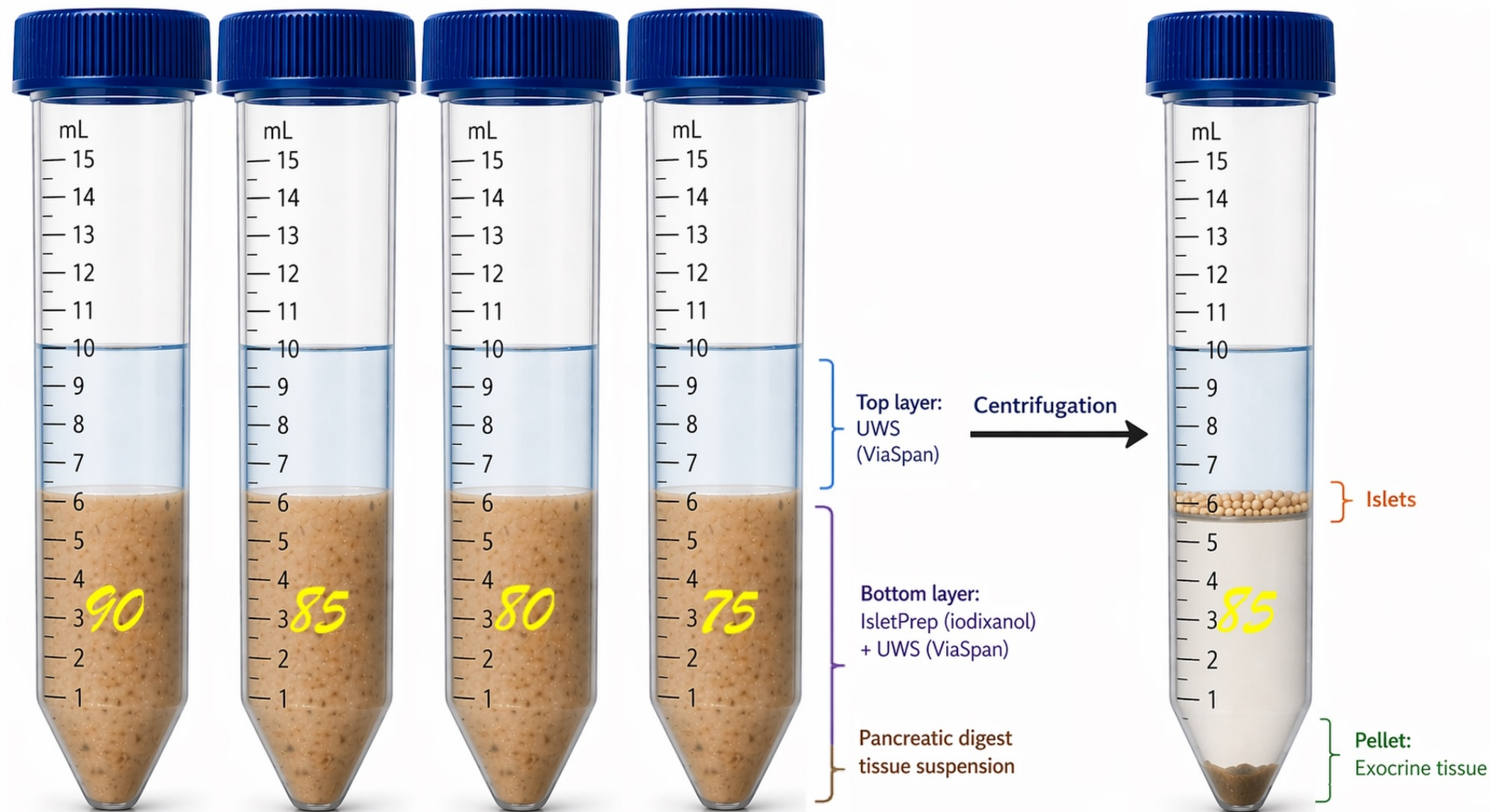
After 5 min centrifugation at 4°C and 500 g, collect islets from the interface

Islet Purification

1-Step Density Gradients • Test Purification • Bulk Purification

- Test Purification
 - A 5% aliquot of the digest was distributed over 4 (-7) small test tubes
 - mixed with IsletPrep solution to prepare the bottoms of 1-step gradients with densities 1090, 1085, 1080, 1075 (1100, 1095, 1072) — topped with UWS
 - Prediction of the optimal gradient density for bulk purification by analysis of interfaces and pellets in the test tubes after centrifugation – aimed at maximum islet recovery at a purity of at least ~70%
- Bulk Purification
 - The digest in 200-450 ml UWS was mixed with IsletPrep, distributed over 10-20 conicals, topped with UWS, and spun 5 min at 500 g , 4°C
 - Purified islets were collected from the interface of the 1-step gradients

Test Purification of Isolated Islets of Langerhans

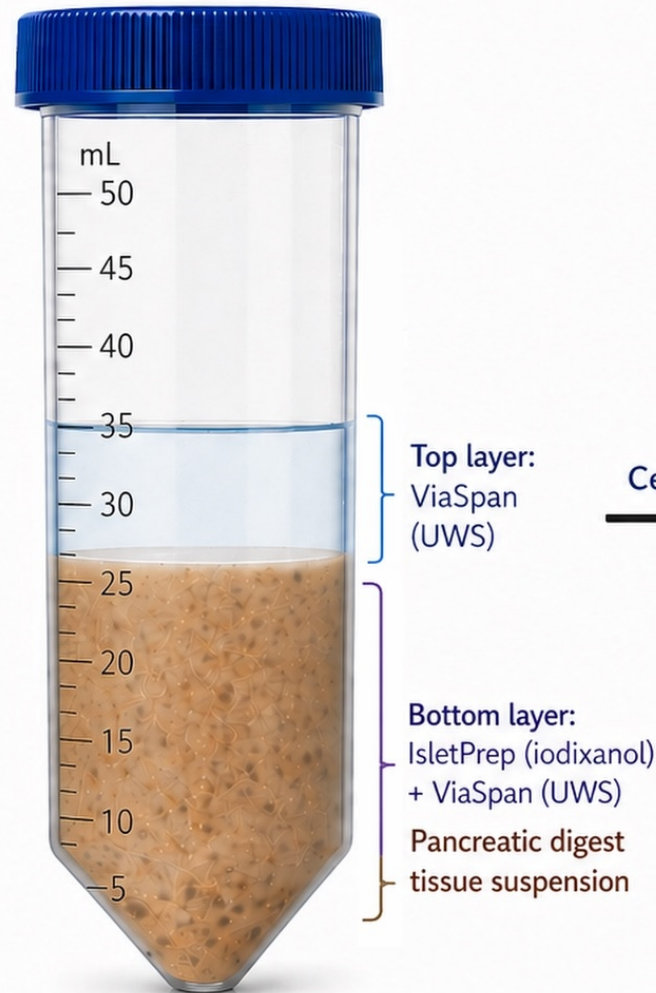


1-Step Density Gradient Centrifugation Using IsletPrep (iodixanol) + UWS (ViaSpan)

Bulk Purification of Isolated Islets of Langerhans

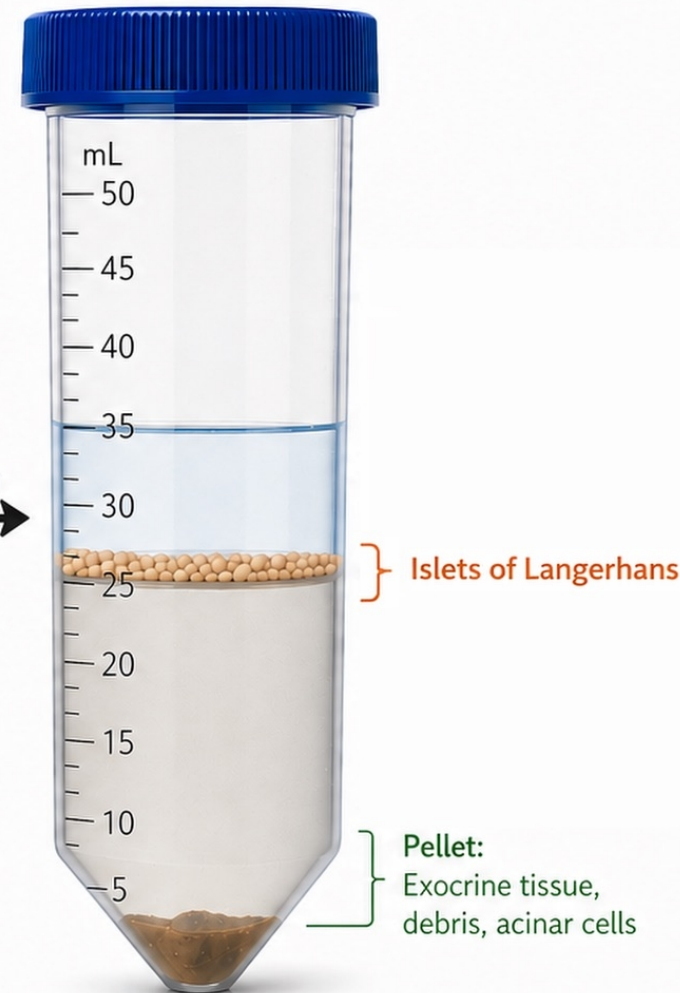
1-Step Density Gradient Centrifugation Using IsletPrep (iodixanol) + ViaSpan (UWS)

BEFORE CENTRIFUGATION
(Loaded Tube)



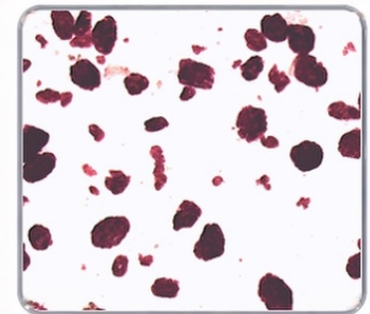
Centrifugation

AFTER CENTRIFUGATION
(Purified Islets)



KEY

-  ViaSpan (UWS)
(Top layer)
-  IsletPrep (iodixanol)
+ ViaSpan (UWS)
(Bottom layer)
-  Islets of Langerhans
(at interface)
-  Pellet (unwanted
tissue and debris)
-  Pancreatic digest
(before centrifugation)



Purified Islets of Langerhans
(Typical appearance)

Islets band at the interface between the top ViaSpan (UWS) layer and the bottom IsletPrep (iodixanol) + ViaSpan (UWS) layer (25 mL mark), while unwanted tissue and debris pellet at the bottom.

IsletPrep Results

Prediction of the optimal density for bulk purification in the 1-step gradient by analyzing the densities of the islets and acinar tissue beforehand in our set of test tubes resulted in a density <1085, 1090, 1080, 1090, 1090, 1090, 1095, 1090 g/L resp. during consecutive experiments (1090 on average).

Islet Recovery	DIGEST	ISLETPREP
IEQs/g (% recovery)	1412 ± 291	1251 ± 181 (106 %)
% MANTLE-ISLETS	33 ± 5	37 ± 14
DIAMETER (μm)	157 ± 11	202 ± 15
% PURITY		74 ± 5

Powerful Tool for Human Islet Purification

Thus, the consistent high efficacy of this simple Iodixanol-UWS IsletPrep gradient: no loss of islets, mantle islets, islet integrity, and a high purity

& the favorable characteristics such as

- the mild hyperosmotic conditions (~ 380 mOsm)
- a low endotoxin content

suggest the IsletPrep gradient to become a powerful new tool for human islet purification.

2000-2001 — Visipaque-Viaspan Clinical Grade Islet Purification...

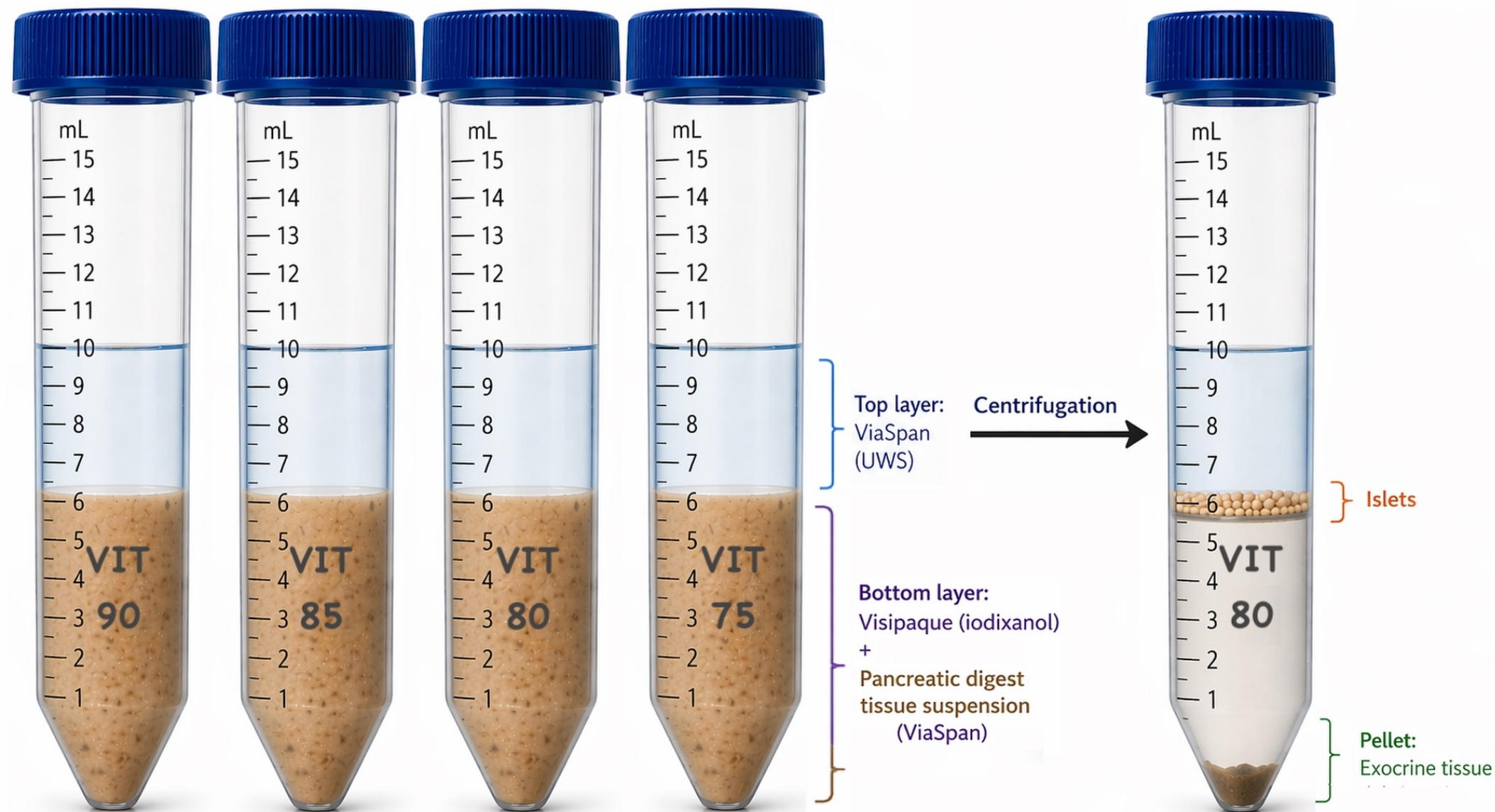
- As GMP production of IsletPrep would not be ready in time for our planned start of work in the GMP Facility, we validated a simpler alternative combining clinical-grade Visipaque and ViaSpan.
- Purification without IsletPrep — that is, without the double-strength UWS component — also seemed an option: simply mixing OptiPrep and UWS had already proven sufficient for purification in animal experimental work in 1998*, indicating that double-strength UWS (i.e. IsletPrep) might not be strictly necessary for our Iodixanol-UWS gradients.

*Islet purification from bench to bedside • @1MEMO_20260623 • Michel van der Burg • Miracles.Media • URL michelvanderburg.com/2026/06/23/

2000-2001 — Visipaque-Viaspan Clinical Grade Islet Purification

- OptiPrep was a research-grade solution of 60% iodixanol, but could be replaced by the clinical-grade Visipaque solution of 65% iodixanol.
- Simply mixing Visipaque (iodixanol) and ViaSpan (UWS) — both commercial, cGMP-certified solutions — would be an elegant alternative for our Iodixanol-UWS gradients.
- During the first months of 2001, the 2000 series of human islet isolations was completed in our research laboratory with a successful pilot of islet purification using Visipaque-ViaSpan.

Test Purification of Isolated Islets of Langerhans

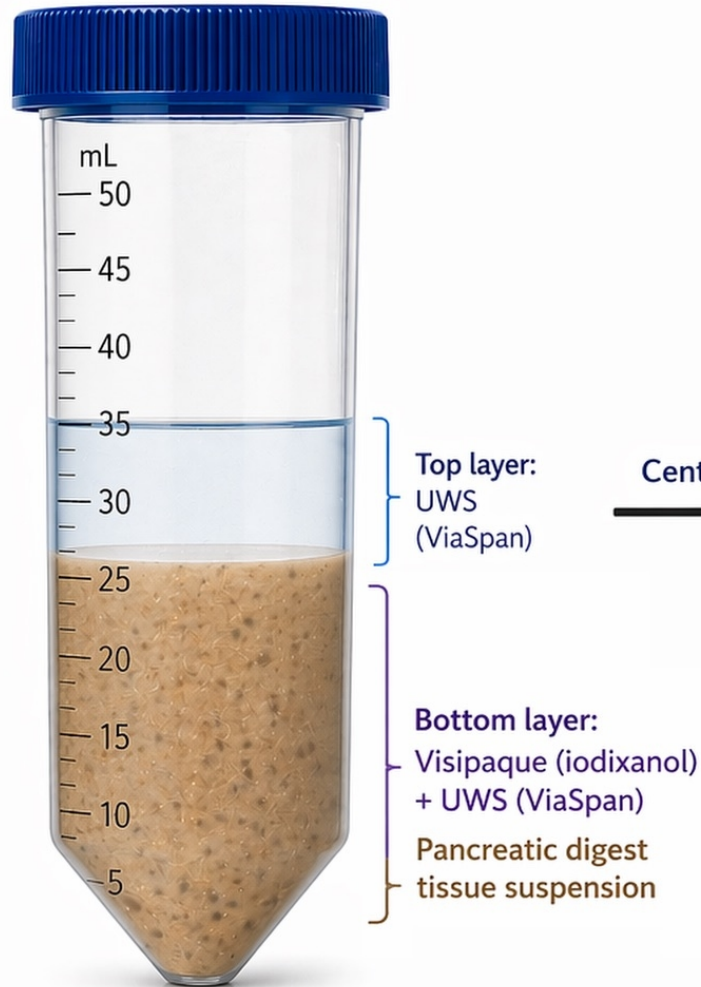


1-Step Density Gradient Centrifugation Using Visipaque (iodixanol) + ViaSpan (UWS)

Bulk Purification of Isolated Islets of Langerhans

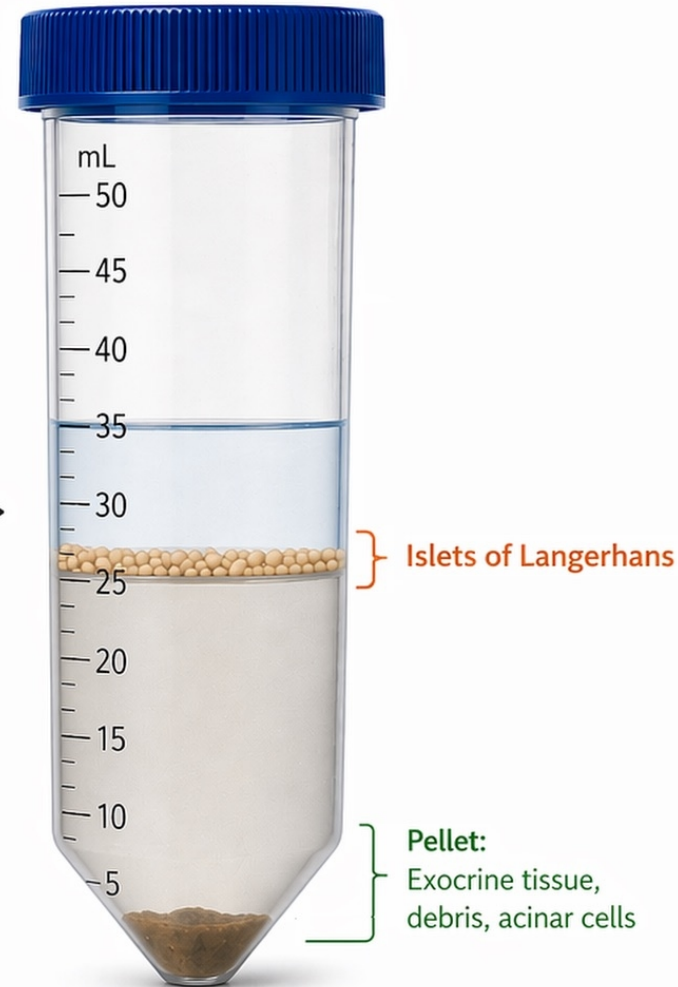
1-Step Density Gradient Centrifugation Using Visipaque (iodixanol) + UWS (ViaSpan)

BEFORE CENTRIFUGATION
(Loaded Tube)



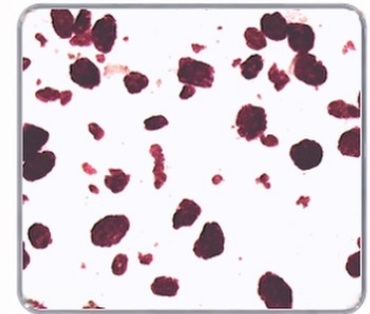
Centrifugation →

AFTER CENTRIFUGATION
(Purified Islets)



KEY

- UWS (ViaSpan) (Top layer)
- Visipaque (iodixanol) + UWS (ViaSpan) (Bottom layer)
- Islets of Langerhans (at interface)
- Pellet (unwanted tissue and debris)
- Pancreatic digest (before centrifugation)



Purified Islets of Langerhans
(Typical appearance)

Islets band at the interface between the top UWS layer and the bottom Visipaque + UWS layer (25 mL mark), while unwanted tissue and debris pellet at the bottom.

VISIPAQUE RESULTS

Test purification performed during islet isolation on a fraction of digested pancreatic tissue (digest) demonstrated a density optimum ≤ 1085 — aimed at maximum islet recovery at a purity of at least $\sim 70\%$.

Final bulk purification of the entire batch demonstrated no loss of islets, mantle-islets (i.e. islets with a rim of pancreatic acinar tissue), islet integrity (diameter), and $\sim 70\%$ purity.

Islet Recovery	DIGEST	VISIPAQUE-VIASPAN
IEQs/g (% recovery)	1437 $\pm$ 15	1681 $\pm$ 55 (118 %)
% MANTLE-ISLETS	18 $\pm$ 13	13 $\pm$ 3
DIAMETER (μ m)	185 $\pm$ 16	184 $\pm$ 8
% PURITY		71 $\pm$ 16

GMP-Compliant Islet Purification

Thus, the simpler alternative combining clinical-grade Visipaque and ViaSpan, matched IsletPrep's results in human islet purification : no loss of islets, mantle islets, islet integrity, and a high purity

Visipaque-ViaSpan's favorable characteristics include :

- the mild hyperosmotic conditions (~315 mOsm calculated)
- the low endotoxin content
- a simple, safe, cGMP certified clinical-grade purification solution

This Visipaque-ViaSpan protocol became the standard, enabling the opening of the LUMC's Clinical Islet Laboratory (Klinisch EilandLaboratorium) in April 2001.

Visipaque-ViaSpan SOPs

Klinisch

Eilandlaboratorium

GMP-documentatie

Revisie 1

02.05.08

Bestemd voor: M.P.M. van der Burg



GMP-documentation Clinical Islet Laboratory LUMC

Procedure report: Human islet isolation

SOP: PR-0010/04

Effective date (yy.mm.dd): 01.12.01

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TEST PURIFICATION & BULK PURIFICATION VISIPAQUE

TEST PURIFICATION & PROCEDURE BULK PURIFICATION						
Visipaque lot:	10129973	Expiry date:	12/01/03	Isolation #	 M	
TEST PURIFICATION				Start time:		
Test densities:	VIT95	VIT90	VIT85	VIT80	VIT75	VIT72
µL VIS/5 mL ViaSpan	940	825	720	615	515	465
Done (✓)						
Top (note +++/+/0/NA; or % if indicated; mantle islets as % of all islets):						
Recovery islets:						
Recovery acinar:						
Purity %:						
Mantle islets %:						
Bottom inspection (note +++/+/0; and mantle-islets as % of all islets in bottom):						
Done (✓)						
Loss islets:						
Mantle islets %:						
Optimal density:						
BULK PURIFICATION						
Procedure (-/-):				Start time:		
COBE	TUBES	Number sessions:	Time start:			
Visipaque(1.3600)	VIS95	VIS90	VIS85	VIS80	VIS75	VIS72
mL VIS / 200mL ViaSpan	37.5	33	28.8	24.6	20.6	18.5
Gradient Type (✓)						
..... 1-Step: Bottom: V95/ V90/ V85/ V80/ V75/ V72/ V.....						
Top: ViaSpan/ different:						
..... 2-Step: Bottom: V95/ V90/ V85/ V80/ V75/ V72/ V.....						
Barrier: V90/ V85/ V80/ V75/ V72/ V.....						
Top: ViaSpan/ different:						
Macroscopic aspect gradient / Comment						
TOTAL VOLUME FRACTIONS WHEN TAKING SAMPLES FOR MORPHOMETRY						
CODE FRACTION 1: INTERFACE: VOLUME (mL):						% Total
CODE FRACTION 2: INTERFACE: VOLUME (mL):						

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GMP-documentation Clinical Islet Laboratory LUMC

Procedure report: Preparation Visipaque-UWS media

SOP: PR-1100/03

Effective date (yy.mm.dd): 01.09.07

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3. 'Test tubes' preparation with Visipaque (VIS)

Label 15-ml tubes VIT95, VIT90, etc. according to column 'Code'

For isolation, at least the following must be available: 1 set of test tubes (VIT90, 85, 80, 75)

Pipette exclusively Visipaque (VIS) into the tubes using an Eppendorf 100-1000 µl Reference pipette, according to column 'µl VIS'.

Gradient Density	µl VIS	VIS Density	ViaSpan / Digest ml	ViaSpan Density	Code	N	Density	°C
1.0950	940	1.3564	5.01	1.046	VIT95			
1.0900	825	1.3564	5.00	1.046	VIT90			
1.0850	720	1.3564	5.01	1.046	VIT85			
1.0800	615	1.3564	5.00	1.046	VIT80			
1.0750	515	1.3564	5.00	1.046	VIT75			
1.0723	465	1.3564	5.02	1.046	VIT72			

Keep tubes at approx. 4°C and use within 1 hour for test purification or density testing
For test purification, add 5 mL digest (in ViaSpan)

4. OPTIONAL: 'Test tubes' density test

Prepare 1-2 series of tubes (Note the number of tubes prepared (excl. for density test) above at N.
Add 5 ml ViaSpan to each tube, close, and mix by swirling.
Take approx. 5 ml of sample using a 10 ml syringe for density measurement (IV-0130).
Record the results (density and temperature) above under Density and °C.

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Visipaque-ViaSpan SOPs · 1MEMO_20260720_1_7 · Michel van der Burg · Illustration of "Procedure report" pages on using Visipaque for "Test tubes" preparation (right) and "Purification" (middle) during human islet isolation, with the pancreatic digest in UWS (ViaSpan), from the 2001 standard operating procedures — the SOPs, now "Obsolete" (i.e. outdated) — from the GMP documentation for the Clinical Islet Laboratory (Klinisch Eilandlaboratorium) at LUMC (Leiden, Netherlands). Translated from Dutch by author, Michel van der Burg

Notes

This work was shared previously with the other islets centers, in personal communication in 2001 — in detail with Jonathan Lakey (Edmonton, Canada), and in summary with Daniel Brandhorst (Giessen, Germany) , José Oberholzer (Geneva, Switzerland) — and with Sue Swift (Leicester, UK) in 2003.

† Acknowledgements — I thank Bjørn Henriksen (International Product Group Manager Density Gradient Media Nycomed / Axis-Shield PoC AS (Oslo, Norway) for our collaboration on the iodixanol and IsletPrep work ; Camillo Ricordi (Director of the Diabetes Research Institute, Miami) and Ramon Poo (BioRep Technologies, Miami) for help in setting up automated islet isolation in our lab ; Amon Wafelman (deputy head of the Interdivisional GMP-Facility, Department of Clinical Pharmacy and Toxicology, LUMC) for collaboration on SOPs and the GMP setup of the clinical islet laboratory ; our Transplant Coordinators Marijke van Gorp and Ruth Dam (EuroTransplant / LUMC) and my co-workers of the Transplant section of the Surgery Department (LUMC) : surgeons Jan Ringers, André Baranski, Xandro Schaapherder for dedicated organ procurement, and the researchers Eelco Bouwman, Annemiek Töns (staff), and Josephine Rijkkelijkhuizen, Gabe Bleeker (students) for collaboration in our 'islet team'.

Citation info : 2000 — Pioneering Automated Islet Isolation & Clinical Grade Purification in the LUMC • 1MEMO_20260720 • Michel van der Burg • Miracles•Media • URL michelvanderburg.com/2026/07/20