

Lot No.: **69F**

Lot-specific information

## **Olerup SSP<sup>®</sup> HLA-Cw\*16**

**Product number:** 101.627-12u – without *Taq* polymerase  
**Lot number:** 69F  
**Expiry date:** 2011-March-01  
**Number of tests:** 12  
**Number of wells per test:** 8  
**Storage - pre-aliquoted primers:** dark at -20°C  
- PCR Master Mix: -20°C  
- Adhesive PCR seals RT  
- Product Insert RT

**This Product Description is only valid for Lot No. 69F.**

### **CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP<sup>®</sup>* HLA-Cw\*16 LOT**

The HLA-Cw\*16 specificity and interpretation tables have been updated for the HLA-Cw alleles described since the previous *Olerup SSP<sup>®</sup>* HLA-Cw\*16 lot was made (**Lot No. 22E**).

The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

<b>Well</b>	<b>5'-primer</b>	<b>3'-primer</b>	<b>rationale</b>
3	Added	Added	Primer added for the Cw*1612 allele.
6	Added	Added	Primer added for the Cw*1611 allele.

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## PRODUCT DESCRIPTION

### HLA-Cw\*16 SSP typing

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the Cw\*160101 to Cw\*1612 alleles.

#### PLATE LAYOUT

Each HLA-Cw\*16 test consists of 8 PCR reactions in an 8 well cut PCR plate.

1	2	3	4	5	6	7	8
---	---	---	---	---	---	---	---

The 8 well PCR plate is marked with 'Cw16' in silver/gray ink.

Well No. 1 is marked with the Lot No. '69F'.

The PCR plates are heat-sealed with a PCR-compatible foil.

**Please note:** When removing each 8 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

#### INTERPRETATION

The interpretation of HLA-Cw\*16 SSP subtypings will be influenced by other HLA-Cw alleles, as primer mixes 2 to 6 amplify non-HLA-Cw\*16 alleles. In addition, primer mix 4 will amplify the B\*350802 and B\*6702 alleles.

#### UNIQUELY IDENTIFIED ALLELES

All the HLA-Cw\*16 alleles, i.e. **Cw\*1601 to Cw\*1612**, recognized by the HLA Nomenclature Committee in January 2009<sup>1</sup> will be amplified by the primers in the HLA-Cw\*16 SSP kit.

The HLA-Cw\*16 primer set cannot distinguish the Cw\*160101 and Cw\*160103 alleles.

<sup>1</sup>HLA-Cw alleles listed on the IMGT/HLA web page 2009-January-16, release 2.24.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

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### RESOLUTION IN HOMO- AND HETEROZYGOTES

The ten phenotypically different HLA-Cw\*16 alleles give rise to eleven amplification patterns that can be combined in 66 homozygous and heterozygous combinations. Fifty-one of these genotypes do not give rise to unique amplification patterns. The different lengths of the specific PCR products generated by primer mixes 3, 6 and 7 were not considered in these calculations.

```
+++++-- 1606,1612 = 1610,1612 = 1611,1612
+++++-- 160401,1609 = 1607,1612
+++++-- 1601,1612 = 1602,160401 = 160401,1612
+++-+-- 160102,1612 = 1602,1612 = 1612,1612
+++++-- 1606,1609 = 1609,1610 = 1609,1611
+++-+-- 1602,1606 = 1602,1610 = 1602,1611
+++-+-- 1601,1609 = 1602,1607 = 1607,1609
+++-+-- 160102,1609 = 1602,1609 = 1609,1609
+++-+-- 160102,1602 = 1602,1602
+++-+-- 160401,1606 = 160401,1611
+++-+-- 1601,160401 = 160102,160401
+++-+-- 1607,1610 = 1607,1611
+++-+-- 1606,1608 = 1608,1610 = 1608,1611
+++-+-- 1601,1606 = 1601,1610 = 1601,1611 = 160102,1606 =
160102,1610 = 160102,1611 = 1606,1610 = 1606,1611
= 1610,1611 = 1611,1611
+++-+-- 1601,1607 = 160102,1607
+++-+-- 1601,1608 = 160102,1608 = 1608,1608
+++-+-- 1601,1601 = 1601,160102
```

1601 = 160101 and 160103

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## SPECIFICITY TABLE

### HLA-Cw\*16 SSP subtyping

Specificities and sizes of the PCR products of the 8 primer mixes used for HLA-Cw\*16 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-Cw*16 alleles	Other amplified HLA Class I alleles <sup>3</sup>
<b>1</b>	205 bp	<b>800 bp</b>	160101-1602, 160401, 1608-1612	
<b>2</b>	340 bp	1070 bp	1602, 1609, 1612	0114, 020201-020203, 020205-0211, 0213-0223, 0307, 0315, 0345, 04010101-040106, 0403-0410, 0412-0420, 0423-0428, 0430-0435, 0437, 05010101-050104, 0503-0523, 06020101-06020102, 060203-0610, 0612-0619, 0707, 0709, 0749, 0810, 120401-1205, 1209, 1221, 1404, 150201-1506, 1508-1513, 1515-1520, 1701-1705, 1801-1803
<b>3<sup>4,5,6</sup></b>	85, 220 bp	<b>800 bp</b>	160401, 1612	0104, 0109, 0205, 0217, 06020101-06020102, 060203, 0603, 0607-0613, 0615-0619, 12030101-1207, 1211-1213, 1215
<b>4<sup>4</sup></b>	140 bp	1070 bp	160101, 160103, 160401, 1606-1608, 1610, 1611	0121, 0212 <sup>weakly</sup> , 0411, 0429, 0436, 080101-0809, 0811-0819, 120201-120303, 120305-120306, 1206-1208, 1210-1220, 140203,

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				1403, 1408, 1410, 1507, 1521 <sup>weakly</sup> , <b>B*350802, B*6702</b>
<b>5</b>	160 bp	<b>800 bp</b>	160101-1602, 1606-1609, 1611, 1612	0753
<b>6<sup>7</sup></b>	130, 160, 210 bp	1070 bp	1606, 1610, 1611	0221
<b>7<sup>4,8</sup></b>	100, 210 bp	1070 bp	1607, 1609	
<b>8<sup>4</sup></b>	135 bp	1070 bp	1608	

<sup>1</sup>Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of HLA-Cw\*16 high resolution SSP typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective length of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers.

PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings.

PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings.

Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

<sup>2</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw\*16 SSP subtyping.

In addition, wells number 3 and 5 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band.

In the presence of a specific amplification the intensity of the control band often decreases.

<sup>3</sup>Due to the sharing of sequence motifs between HLA-Cw alleles non-HLA-Cw\*16 alleles will be amplified by primer mixes 2 to 6. In addition, the B\*350802 and B\*6702 alleles will be amplified by primer mix 4.

<sup>4</sup>Short specific PCR fragments are less intense and not as sharp as longer specific bands.

<sup>5</sup>Primer mix 3 may give a lower yield of specific PCR product than the other Cw\*16 primer mixes.

<sup>6</sup>Primer mix 3: Specific PCR fragment of 85 bp in the Cw\*1612 allele. Specific PCR fragment of 220 bp in the Cw\*160401 and in the Cw\*0104, 0109, 0205, 0217, 06020101 to 06020102, 060203, 0603, 0607 to 0613, 0615 to 0619, 12030101 to 1207, 1211 to 1213 and 1215 alleles.

<sup>7</sup>Primer mix 6: Specific PCR fragment of 130 bp in the Cw\*1611 and in the Cw\*0221 allele. Specific PCR fragment of 160 bp in the Cw\*1610 allele. Specific PCR fragment of 210 bp in the Cw\*1606 allele.

<sup>8</sup>Primer mix 7: Specific PCR fragment of 100 bp in the Cw\*1609 allele. Specific PCR fragment of 210 bp in the Cw\*1607 allele.

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<b>INTERPRETATION TABLE</b>								
<b>HLA-Cw*16 SSP subtyping</b>								
<b>Amplification patterns of the HLA-Cw*1601 to 1612 alleles</b>								
	<b>Well<sup>5</sup></b>							
	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
<b>Length of spec.</b>	<b>205</b>	<b>340</b>	<b>85</b>	<b>140</b>	<b>160</b>	<b>130</b>	<b>100</b>	<b>135</b>
<b>PCR product</b>			<b>220</b>			<b>160</b>	<b>210</b>	
						<b>210</b>		
<b>Length of int.</b>	<b>800</b>	<b>1070</b>	<b>800</b>	<b>1070</b>	<b>800</b>	<b>1070</b>	<b>1070</b>	<b>1070</b>
<b>pos. control<sup>1</sup></b>								
<b>5'-primer(s)<sup>2</sup></b>	<b>361</b>	<b>1<sup>st</sup> I</b>	<b>256</b>	<b>201</b>	<b>419</b>	<b>113</b>	<b>244</b>	<b>126</b>
	5'-AgT <sup>3'</sup>	5'-CgA <sup>3'</sup>	5'-ACg <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-gTC <sup>3'</sup>	5'-CCA <sup>3'</sup>	5'-CgC <sup>3'</sup>	5'-ggA <sup>3'</sup>
			<b>361</b>			<b>368</b>	<b>369</b>	
			5'-AgT <sup>3'</sup>			5'-gTC <sup>3'</sup>	5'-TAC <sup>3'</sup>	
						<b>418</b>		
						5'-Agg <sup>3'</sup>		
<b>3'-primer(s)<sup>3</sup></b>	<b>527</b>	<b>302</b>	<b>302</b>	<b>302</b>	<b>539</b>	<b>201</b>	<b>302</b>	<b>220</b>
	5'-CCg <sup>3'</sup>	5'-ggT <sup>3'</sup>	5'-ggT <sup>3'</sup>	5'-ggC <sup>3'</sup>	5'-TCT <sup>3'</sup>	5'-CTT <sup>3'</sup>	5'-ggT <sup>3'</sup>	5'-CgA <sup>3'</sup>
			<b>538</b>			<b>539</b>	<b>539</b>	
			5'-CCA <sup>3'</sup>			5'-TCT <sup>3'</sup>	5'-TCT <sup>3'</sup>	
<b>Well No.</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>
<b>HLA-Cw allele<sup>4</sup></b>								
<b>*160101, 160103</b>	<b>1</b>			<b>4</b>	<b>5</b>			
<b>*160102</b>	<b>1</b>				<b>5</b>			
<b>*1602</b>	<b>1</b>	<b>2</b>			<b>5</b>			
<b>*160401</b>	<b>1</b>		<b>3</b>	<b>4</b>				
<b>*1606</b>				<b>4</b>	<b>5</b>	<b>6</b>		
<b>*1607</b>				<b>4</b>	<b>5</b>		<b>7</b>	
<b>*1608</b>	<b>1</b>			<b>4</b>	<b>5</b>			<b>8</b>
<b>*1609</b>	<b>1</b>	<b>2</b>			<b>5</b>		<b>7</b>	
<b>*1610</b>	<b>1</b>			<b>4</b>		<b>6</b>		
<b>*1611</b>	<b>1</b>			<b>4</b>	<b>5</b>	<b>6</b>		
<b>*1612</b>	<b>1</b>	<b>2</b>	<b>3</b>		<b>5</b>			
<b>*0104, 0109, 0611, 120304</b>			<b>3</b>					
<b>Well No.</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>

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Length of spec.	205	340	85	140	160	130	100	135
PCR product			220			160	210	
						210		
Well No.	1	2	3	4	5	6	7	8
*0114, 020201-020203, 020205-0204, 0206-0211, 0213-0216, 0218-0220, 0222, 0223, 0307, 0315, 0345, 04010101-040106, 0403-0410, 0412-0420, 0423-0428, 0430-0435, 0437, 050101-050104, 0503-0523, 0604-0606, 0614, 0707, 0709, 0749, 0810, 1209, 1221, 1404, 150201-1506, 1508-1513, 1515-1520, 1701-1705, 1801-1803	2							
*0121, 0411, 0429, 0436, 080101-0809, 0811-0819, 120201-120203, 1208, 1210, 1214, 1216-1220, 140203, 1403, 1408, 1410, 1507				4				
*0205, 0217, 06020101-0603, 0607-0610, 0612, 0613, 0615-0619, 120401-1205		2	3					
*0212, 1521				w				
*0221		2			6			
*0753					5			
*12030101-120303, 120305-120306, 1206, 1207, 1211-1213, 1215			3	4				
HLA-Cw allele <sup>4</sup>								
Well No.	1	2	3	4	5	6	7	8
B*350802, B*6702				4				
Well No.	1	2	3	4	5	6	7	8

<sup>1</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-Cw\*16 SSP subtyping.

In addition, wells number 3 and 5 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band.

<sup>2</sup>The nucleotide position, in the 2<sup>nd</sup> or 3<sup>rd</sup> exon or the 1<sup>st</sup> intron, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>3</sup>The nucleotide position, in the 2<sup>nd</sup> or 3<sup>rd</sup> exon, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the

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[www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>4</sup>The HLA-Cw\*1603 nucleotide sequence has been shown to be identical to Cw\*1403.

The HLA-Cw\*160402 nucleotide sequence has been shown to be identical to Cw\*160401.

The HLA-Cw\*1605 nucleotide sequence has been shown to be identical to Cw\*160401.

<sup>5</sup>Primer mix 3: Specific PCR fragment of 85 bp in the Cw\*1612 allele. Specific PCR fragment of 220 bp in the Cw\*160401 and in the Cw\*0104, 0109, 0205, 0217, 06020101 to 06020102, 060203, 0603, 0607 to 0613, 0615 to 0619, 12030101 to 1207, 1211 to 1213 and 1215 alleles.

<sup>7</sup>Primer mix 6: Specific PCR fragment of 130 bp in the Cw\*1611 and in the Cw\*0221 allele. Specific PCR fragment of 160 bp in the Cw\*1610 allele. Specific PCR fragment of 210 bp in the Cw\*1606 allele.

<sup>8</sup>Primer mix 7: Specific PCR fragment of 100 bp in the Cw\*1609 allele. Specific PCR fragment of 210 bp in the Cw\*1607 allele.

'w' might be weakly amplified.



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<b>CELL LINE VALIDATION SHEET</b>													
<b>HLA-Cw*16 SSP primer set</b>													
					<b>Well</b>								
					<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	
					Prod. No.:	200729401	200729402	200957603	200729404	200729405	200957606	200729407	200957608
<b>IHWC cell line</b>			<b>Cw*</b>										
1	9001	SA	*0702		-	-	-	-	-	-	-	-	
2	9280	LK707	*0701	*1505	-	+	-	-	-	-	-	-	
3	9011	E4181324	*1202		-	-	-	+	-	-	-	-	
4	9275	GU373	*0304	*0401	-	+	-	-	-	-	-	-	
5	9009	KAS011	*0602		-	+	+	-	-	-	-	-	
6	9353	SM	*0304	*0702	-	-	-	-	-	-	-	-	
7	9020	QBL	*0501		-	+	-	-	-	-	-	-	
8	9007	DEM	*0602		-	+	+	-	-	-	-	-	
9	9026	YAR	*1203		-	-	+	+	-	-	-	-	
10	9107	LKT3	*0102		-	-	-	-	-	-	-	-	
11	9051	PITOUT	*1601		+	-	-	+	+	-	-	-	
12	9052	DBB	*0602		-	+	+	-	-	-	-	-	
13	9004	JESTHOM	*0102		-	-	-	-	-	-	-	-	
14	9071	OLGA	*0102	*0304	-	-	-	-	-	-	-	-	
15	9075	DKB	*0304		-	-	-	-	-	-	-	-	
16	9037	SWEIG007	*0202		-	+	-	-	-	-	-	-	
17	9282	CTM3953540	*0303	*0701	-	-	-	-	-	-	-	-	
18	9257	32367	*0102	*0705	-	-	-	-	-	-	-	-	
19	9038	BM16	*0701		-	-	-	-	-	-	-	-	
20	9059	SLE005	*0304		-	-	-	-	-	-	-	-	
21	9064	AMALA	*0303		-	-	-	-	-	-	-	-	
22	9056	KOSE	*1203		-	-	+	+	-	-	-	-	
23	9124	IHL	*0102	*1502	-	+	-	-	-	-	-	-	
24	9035	JBUSH	*1203		-	-	+	+	-	-	-	-	
25	9049	IBW9	*0802		-	-	-	+	-	-	-	-	
26	9285	WT49	*0701		-	-	-	-	-	-	-	-	
27	9191	CH1007	*0704	*1505	-	+	-	-	-	-	-	-	
28	9320	BEL5GB	*0501	*1601	+	+	-	+	+	-	-	-	
29	9050	MOU	*1601		+	-	-	+	+	-	-	-	
30	9021	RSH	*1701		-	+	-	-	-	-	-	-	
31	9019	DUCAF	*0501		-	+	-	-	-	-	-	-	
32	9297	HAG	*1701	*1703	-	+	-	-	-	-	-	-	
33	9098	MT14B	*0304		-	-	-	-	-	-	-	-	
34	9104	DHIF	*1203		-	-	+	+	-	-	-	-	
35	9302	SSTO	*0501		-	+	-	-	-	-	-	-	
36	9024	KT17	*0303	*0401	-	+	-	-	-	-	-	-	
37	9065	H-HKB	*0702		-	-	-	-	-	-	-	-	
38	9099	LZL	*0303		-	-	-	-	-	-	-	-	
39	9315	CML	*0202	*0701	-	+	-	-	-	-	-	-	
40	9134	WHONP199	*0602		-	+	+	-	-	-	-	-	
41	9055	H0301	*0802		-	-	-	+	-	-	-	-	
42	9066	TAB089	*0102		-	-	-	-	-	-	-	-	
43	9076	T7526	*0102	*0801	-	-	-	+	-	-	-	-	
44	9057	TEM	*1203		-	-	+	+	-	-	-	-	
45	9239	SHJO	*0602	*1701	-	+	+	-	-	-	-	-	
46	9013	SCHU	*0702		-	-	-	-	-	-	-	-	
47	9045	TUBO	*0704	*1502	-	+	-	-	-	-	-	-	
48	9303	TER-ND	*0401	*1601	+	+	-	+	+	-	-	-	



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## CERTIFICATE OF ANALYSIS

### **Olerup SSP<sup>®</sup> HLA-Cw\*16 SSP**

**Product number:** 101.627-12u – without *Taq* polymerase  
**Lot number:** 69F  
**Expiry date:** 2011-March-01  
**Number of tests:** 12  
**Number of wells per test:** 8

#### **Well specifications:**

<b>Well No.</b>	<b>Production No.</b>
1	2007-294-01
2	2007-294-02
3	2009-576-03
4	2007-294-04
5	2007-294-05
6	2009-576-06
7	2007-294-07
8	2009-576-08

The specificity of each primer solution of the HLA-Cw\*16 primer set has been tested against 48 well characterized cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solutions 6, 7 and 8 were available. The specificity of the primers in primer solutions 6 and 7 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 8 it was only possible to test the 3'-primer, the 5'-primer was not possible to test. In primer solution 6 and 7 two respective one of the 5'-primers were not possible to test. Additional primers in primer solution 3 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer.

**Results:** No false positive or false negative amplifications were obtained.

**Date of approval:** 2009-May-26

**Approved by:**

**Quality Control, Supervisor**

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## Declaration of Conformity

**Product name:** *Olerup* SSP® HLA-Cw\*16  
**Product number:** 101.627-12u  
**Lot number:** 69F

**Intended use:** HLA-Cw\*16 high resolution histocompatibility testing

**Manufacturer:** *Olerup* SSP AB  
Hasselstigen 1  
SE-133 33 Saltsjöbaden, Sweden  
**Phone:** +46-8-717 88 27  
**Fax:** +46-8-717 88 18

We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2000 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex III, as transposed into the national laws of the Member States of the European Union.

The Technical Documentation File is maintained at *Olerup* SSP AB, Hasselstigen 1, SE-133 33 Saltsjöbaden, Sweden.

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Saltsjöbaden, Sweden  
2009-May-26

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