STEC EQA-13 2024-2025

Dear Participant,

Welcome to the thirteenth External Quality Assessment (EQA-13) scheme for typing of STEC in 2024-2025.

NOTE: New virulence gene esta (STa).

If you are using WGS, please read the WGS part of the submission protocol thoroughly before starting your analysis. This year, you are required to use a specific strain/sequence when reporting allele differences/SNP distances.

Please note that most of the fields must be filled in before the submission can be completed. You can write any comments at the end of the form. If you have any questions, please feel free to contact us at ecoli.eqa@ssi.dk.

To begin, please fill in your country, laboratory name, and LAB_ID.

The available options in this participation form include:

- Provide your email to receive a link with your answers. The email containing the link will be sent after pressing "Finish" on the last slide of the survey.

- Open the windows in full screen for the best survey format.

- If the survey is closed before completion, your answers will be saved, and you can return to the survey using the same link.

Note: After pressing "Finish," you will not be able to review your results.

1. Country

- (1) 🗆 Australia
- (2) 🗆 Austria
- (3) 🗆 Belgium
- (4) 🗆 Bulgaria
- (5) 🗆 Canada
- (6) 🗆 Croatia
- (7) 🗆 Czech Republic
- (8) 🗆 Denmark
- (9) 🗆 Estonia
- (10) 🗆 Finland
- (11) 🗆 France
- (12) 🗆 Germany
- (13) 🗆 Greece
- (14) 🗆 Hungary
- (15) 🗆 Iceland
- (16) 🗆 Ireland
- (17) 🗆 Italy
- (18) 🗆 Israel
- (19) 🗆 Latvia
- (20) 🗆 Lithuania
- (21) 🗆 Luxembourg
- (22) 🗆 Malta
- (23) 🗆 México
- (24) 🗆 Montenegro
- (25) 🗆 New Zealand
- (26) 🗆 Norway
- (27) 🗆 Paraguay
- (28) 🗆 Poland
- (29) 🗆 Portugal

- (30) 🗆 Romania
- (31) \Box Scotland, UK
- (32) 🗆 Slovakia
- (33) 🗆 Slovenija
- (34) 🗆 South Africa
- (35) 🗆 Spain
- (36) 🗆 Sweden
- (37) 🗆 The Netherlands
- (38) 🗆 Turkey
- (39) 🗆 United Kingdom
- (40) 🗆 United States of American

2. Institute name

3. Laboratory name

4. Laboratory ID

Consisting of country code (two letters) Lab ID on the vial e.g DK_SSI

5. E-mail		

6. STEC EQA-13 Strain ID's Please enter the isolate ID(4 digits)

We recommend to print this page out!

To have the overview of isolate ID's and isolate No. 1-12, it will make the work easier.

Strain1	
Strain2	
Strain3	
Strain4	
Strain5	
Strain6	
Strain7	
Strain8	

Strain9	
Strain10	
Strain11	
Strain12	

7. Serotyping and virulence gene determination of STEC

8. Submitting results

- (1)
 □ Submit serotyping/virulence gene determination results
- (2) \Box Did not participate in the serotyping or virulence determination part(s) Go to 21

9. Submitting results - Serotyping

- (1) \Box Both O group and H type Go to 10
- (2) \Box Only O Group Go to 10
- (3) \Box Only H type Go to 12
- (4) \Box Did not participate in serotyping Go to 14

10. Results for serotyping (O Group) please type the number of O Group by using (1-188) Non Typable: 7777, Rough: 8888, Not done: 9999

Strain1 Strain2 Strain3 Strain4 Strain5 Strain6 Strain7 Strain8 Strain9 Strain10

O Group

Strain11	
Strain12	

11.Please specify the method used: Phenotypic or molecular (PCR based, WGS based)

	Phenotypic	PCR based	WGS based
Method:	(1) 🗆	(2) 🗆	(3) 🗆

12. Results for serotyping (H Type)please type the number of H Type by using (1-56)H-: 6666, Non Typable: 7777, Not done: 9999

Н Туре

Strain1	
Strain2	
Strain3	
Strain4	
Strain5	

Strain6	
Strain7	
Strain8	
Strain9	
Strain10	
Strain11	
Strain12	

13. Please specify the method used: Phenotypic or molecular (PCR based, WGS based)

	Phenotypic	PCR based	WGS based
Method:	(1) 🗆	(2) 🗆	(3) 🗆

14. Submitting results - Virulence gene determination

(1) Dubmit Virulence gene determination data (*eae, aggR, esta* (STa), stx1, stx2 or subtyping)

(2) \Box Did not participate in the Virulence gene determination *eae*, *aggR*, *esta* (STa) stx1a, stx2 or subtyping). – Go to 21

15. Please specify the method used for the virulence gene determination (incl. subtyping):

	WGS – Go to 17	Other – Go to 16
Method:	(1) 🗆	(2) 🗆

16. If another method is used please describe in detail your method:

17. Results for Virulence gene determination please use 1 for detected and 0 for not detected, Not done: 9999

	eae	aggR	<i>esta</i> (STa)	stx1	stx2
Strain1					
Strain2					
Strain3					
Strain4					

Strain5	 	 	
Strain6	 	 	
Strain7	 	 	
Strain8	 	 	
Strain9	 	 	
Strain10	 	 	
Strain11	 	 	
Strain12	 	 	

18. Submitting results - subtyping results

- (1) 🗆 Submit subtyping data
- (2) \Box Did not participate in subtyping Go to 21

19. Results for subtyping

Subtyping of stx1, select variant (stx1a, stx1c, stx1d)

All isolates have to be subtyped regardless of the results of the initial screening. "Not done/ND" will by default be evaluated as an incorrect result.

	stx1a	stx1c	stx1d	stx1a; stx1c	stx1a; stx1d	stx1c; stx1d	Negative	ND
Strain1	(1)	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain2	(1)	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain3	(1) 🗆	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain4	(1)	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain5	(1) 🗆	(2)	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain6	(1)	(2)	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain7	(1) 🗆	(2)	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain8	(1) 🗆	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain9	(1) 🗆	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain10	(1)	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆
Strain11	(1)	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6) 🗆	(7) 🗆	(8) 🗆

Strain12	(1) 🗆	(2) 🗆	(3) 🗆	(4) 🗆	(5) 🗆	(6)	(7) 🗆	(8) 🗆
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20. Subtyping of stx2 select variant (stx2a, stxb, stx2c, stx2d, stx2e, stx2f, stx2g) All isolates have to be subtyped regardless of the results of the initial screening. "ND" will by default be evaluated as an incorrect result.

	stx2a	stx2b	stx2c	stx2d	stx2e	stx2f	stx2g	stx2a; stx2b	stx2a; stx2c	stx2a; stx2d	stx2a; stx2e	stx2a; stx2g	stx2b; stx2c	stx2b; stx2d	stx2b; stx2g	stx2c; stx2d	stx2c; stx2e	stx2c; stx2g	stx2d; stx2e	stx2d; stx2g	stx2e; stx2f	stx2a; stx2b; stx2c	stx2a; stx2c; stx2d	stx2b; stx2c; stx2d	stx2a; stx2b; stx2c; stx2	Negative	ND
Strain1																											
Strain2																											
Strain3																											
Strain4																											
Strain5																											
Strain6																											
Strain7																											

Strain8												
Strain9												
Strain10												
Strain11												
Strain12												

21. Submitting Cluster results

- (1)
 □ Cluster analyses based on PFGE and/or WGS
- (2) \Box Did not participate in the Cluster part Go to 116

22. Submitting Cluster analysis results

- (1) \Box Cluster analysis based on PFGE Go to 23
- (2) \Box Do not wish to submit any cluster results based on PFGE analysis Go to 26

23. Cluster analysis based on PFGE data

24. Please list the ID for the isolate included in the cluster of closely related isolate detected by PFGE results (bands >33 kb): please use semicolon (;) to separate the ID's

25. Xbal - Total number of bands (>33kb) in a cluster strain

26. Submitting Cluster results

- (1) \Box Cluster analysis based on WGS data Go to 27
- (2) \Box Do not wish to submit any cluster results based on WGS data Go to 116

27. Cluster analysis based on WGS data

28. Please select the analysis used to detect the cluster using WGS The results of the cluster detection can only be reported once (main analysis). If more than one analysis is performed please report later in this submission

- (1) \Box SNP based Go to 30
- (2) \Box Allele based Go to 37
- (3) 🗆 Other Go to 29

29. If another analysis is used please describe in detail your approach (including: assembler, number of loci, variant caller, read mapper or reference ID ect.)

- Go to 44

31. Please select the approach used for the SNP analysis

(1) \Box Reference based – Go to 32

(2) \Box Assembly based – Go to 35

32. Reference genome used:

Preferable use EQA strain0018 (downloaded sequences) as reference. Otherwise indicate Multi-locus Sequence Type (e.g. ST8) and identification of the used reference.

33. Please indicate the read mapper used (e.g. BWA, Bowtie2)

34. Please indicate the variant caller used (e.g. SAMtools, GATK)

35. Please indicate the assembler used (e.g. SPAdes, Velvet)

36. Please specify the variant caller used (e.g. NUCMER)

37. Please select tools used for the allele analysis

- (1) \Box BioNumerics Go to 39
- (2) \Box SeqSphere Go to 39
- (3) 🗆 Enterobase Go to 39
- (4) 🗆 Other Go to 38

38. If another tool is used please enter here:

39. Please indicate allele calling method:

- (1) \Box Assembly based and mapping based Go to 40
- (2) \Box Only assembly based Go to 40
- (3) \Box Only mapping based Go to 41

40. Please indicate the assembler used (e.g. SPAdes, Velvet)

41. Please select scheme used for the allele analysis

- (1) \Box Applied Maths (wgMLST) Go to 43
- (2) D Applied Maths (cgMLST/Enterobase) Go to 43
- (3) 🗆 Enterobase (cgMLST) Go to 43
- (4) \Box Other Go to 42

42. If another scheme (e.g. in-house) is used, please give a short description

43. Please report the number of loci in the used allelic scheme

Cluster detected by analysis on data derived from WGS

On this page you have to report the results for the cluster detected by the selected analysis (e.g. SNP based). If another additional analysis (e.g. allele based or another SNP based analysis) is performed please report results later, but you will not be asked to submit the ID's for isolates in the cluster detected with the additional analysis.

44. Please list the ID for the strains included in the cluster of closely related strains detected by WGS: please use semicolon (;) to separate the ID's This includes the 12 test strains and the 8 provided sequences (20 in total). For the provided sequences write the numbers like: 0013, 0014, 0015, 0016 ect.

45. Report the ID, part of the cluster (yes/no), and SNP distance/allele difference Please use 9999 for not analyzed

	ID	Cluster (Yes/No)	AD/SNP
Strain1		O (Yes)O (No)	
Strain2		O (Yes)O (No)	
Strain3		O (Yes)O (No)	
Strain4		O (Yes)O (No)	
Strain5		O (Yes)O (No)	
Strain6		(Yes)(No)	
Strain7		(Yes)(No)	
Strain8		(Yes)(No)	
Strain9		O (Yes)O (No)	
Strain10		O (Yes)O (No)	
Strain11		O (Yes)	

	0	(No)	
Strain12	 0 0	(Yes) (No)	

46. For each ID report: part of the cluster (yes/no), QC status (A/B/C), QC comment and SNP distance/allele difference

QC status:

Please select the QC status that fits with your assessment of the strain A = Acceptable quality, B = Quality only acceptable for outbreak situations (less good quality), C = Not acceptable quality - strain not analyzed

Distance:

Please use 9999 for not analyzed

	Clu	ster (Yes/No)	QC (A/B/C)		QC comment	AD/SNP		
Strain0013	0	(Yes)	0	(A)				
	0	(No)	0	(B)				
			0	(C)				
Strain0014	0	(Yes)	0	(A)				
	О	(No)	О	(B)				
			0	(C)				
Strain0015	0	(Yes)	0	(A)				
	0	(No)	0	(B)				
			0	(C)				
Strain0016	0	(Yes)	0	(A)				
	0	(No)	0	(B)				
			0	(C)				
Strain0017	0	(Yes)	0	(A)				
	0	(No)	0	(B)				

			0	(C)	
Strain0018	0	(Yes)	0	(A)	
	0	(No)	0	(B)	
			0	(C)	
Strain0019	0	(Yes)	0	(A)	
	0	(No)	0	(B)	
			0	(C)	
Strain0020	0	(Yes)	0	(A)	
	0	(No)	0	(B)	
			0	(C)	

47. (Optional) Would you like to add additional information for the strains? e.g. serotype or sequence type (ST)

- (1) O Yes
- (2) **O** No Go to 48

	Serotype	Subtype	Sequence type (ST)
Strain1			
Strain2			
Strain3			
Strain4			
Strain5			

Strain6	 	
Strain7	 	
Strain8	 	
Strain9	 	
Strain10	 	
Strain11	 	
Strain12	 	
Strain0013	 	
Strain0014	 	
Strain0015	 	
Strain0016	 	
Strain0017	 	

Strain0018	 	
Strain0019	 	
Strain0020	 	

48. Would you like to add results performed with another additional analysis on the data derived from the WGS?

e.g. if SNP based results are submitted you can also report allele based results or results from a second SNP analysis

- (1) 🗆 Yes Go to 49
- (2) \Box No Go to 86

49. Please select the additional analysis used on data derived from WGS

- (1) \Box SNP based Go to 51
- (2) \Box Allele based Go to 58
- (3) 🗆 Other Go to 50

50. If another analysis is used please describe in detail your approach (including: assembler, number of loci, variant caller, read mapper or reference ID ect.)

51. Please report the used SNP-pipeline (reference if publicly available or in-house pipeline)

52. Please select the approach used for the SNP analysis

(1) \Box Reference based – Go to 53

(2) \Box Assembly based – Go to 56

53. Reference genome used: (preferable use EQA strain0018, downloaded sequences as reference). Otherwise indicate Multi-locus Sequence Type (e.g. ST8) and isolate ID

54. Please indicate the read mapper used (e.g. BWA, Bowtie2)

55. Please indicate the variant caller used (e.g. SAMtools, GATK)

56. Please indicate the assembler used (e.g. SPAdes, Velvet)

57. Please specify the variant caller used (e.g. NUCMER)

58. Please select tool used for the allele analysis

(1) \Box BioNumerics – Go to 60

- (2) \Box SeqSphere Go to 60
- (3) 🗆 Enterobase Go to 60

59. If another tool is used please list here:

60. Please indicate allele calling method:

- (1) \Box Assembly based and mapping based Go to 61
- (2) \Box Only assembly based Go to 61
- (3) \Box Only mapping based Go to 62

61. Please indicate the assembler used (e.g. SPAdes, Velvet)

62. Please select scheme used for the allele analysis

- (1) \Box Applied Maths (wgMLST) Go to 64
- (2) D Applied Maths (cgMLST/Enterobase) Go to 64
- (3) 🗆 Enterobase (cgMLST) Go to 64
- (4) 🗆 Other Go to 63

63. If another scheme (e.g. in-house) is used, please give a short description

64. Please report the number of loci in the used allelic scheme

65. Additional analysis on data derived from WGS

66. Results for an additional cluster analysis. Reporting allele differences /SNP distances to strain0018 (as downloaded sequence) (e.g. SNP or Allele based) Please use 9999 for not analysed

	Distance/difference (e.g. SNP/allele) to the strain0018 (downloaded sequence)
Strain1	
Strain2	
Strain3	
Strain4	

Strain5	
Strain6	
Strain7	
Strain8	
Strain9	
Strain10	
Strain11	
Strain12	

Strain0013 (as downloaded sequence)

Strain0014 (as downloaded sequence)

Strain0015 (as downloaded sequence)

Strain0016 (as downloaded sequence)

Strain0017 (as downloaded sequence)

Strain0018 (as downloaded sequence)

Strain0019 (as downloaded sequence)

Strain0020 (as downloaded sequence)

67. Would you like to add results performed with a third analysis on the data derived from the WGS?

e.g. if SNP based results are submitted you can also report allele based results or results from a second SNP analysis

- (1) 🗆 Yes Go to 68
- (2) \Box No Go to 86

68. Please select the third analysis used on data derived from WGS

- (1) \Box SNP based Go to 70
- (2) \Box Allele based Go to 77
- (3) 🗆 Other Go to 69

69. If another analysis is used please describe in detail your approach (including: assembler, number of loci, variant caller, read mapper or reference ID ect.)

70. Please report the used SNP-pipeline (reference if publicly available or in-house pipeline)

71. Please select the approach used for the SNP analysis

- (1) \Box Reference based Go to 72
- (2) \Box Assembly based Go to 75

72. Reference genome used: (preferable use EQA strain0018, downloaded sequences as reference) Otherwise indicate Multi-locus Sequence Type (e.g. ST8) and isolate ID

73. Please indicate the read mapper used (e.g. BWA, Bowtie2)

74. Please indicate the variant caller used (e.g. SAMtools, GATK)

75. Please indicate the assembler used (e.g. SPAdes, Velvet)

76. Please specify the variant caller used (e.g. NUCMER)

77. Please select tool used for the allele analysis

- (1) \Box BioNumerics Go to 79
- (2) \Box SeqSphere Go to 79
- (3) 🗆 Enterobase Go to 79
- (4) 🗆 Other Go to 78

78. If another tool is used please enter here:

79. Please indicate allele calling method:

- (1) \Box Assembly based and mapping based Go to 80
- (2) \Box Only assembly based Go to 80
- (3) \Box Only mapping based Go to 80

80. Please indicate the assembler used (e.g. SPAdes, Velvet)

81. Please select scheme used for the allele analysis

- (1) D Applied Maths (wgMLST) Go to 83
- (2) 🗆 Applied Maths (cgMLST/Enterobase) Go to 83
- (3) 🗆 Enterobase (cgMLST) Go to 83
- (4) 🗆 Other Go to 82

82. If another scheme (e.g. in-house) is used, please give a short description

83. Please report the number of loci in the used allelic scheme

84. Third analysis on data derived from WGS

85. Results for the third cluster analysis. Reporting allele differences /SNP distances to strain0018 (as downloaded sequence) (e.g. SNP or Allele based) Please use 9999 for not analysed

	Distance/difference (e.g. SNP/allele) to the strain0018 (downloaded sequence)
Strain1	
Strain2	
Strain3	
Strain4	
Strain5	
Strain6	
Strain7	
Strain8	
Strain9	
Strain10	

Strain11	
Strain12	
Strain0013 (as downloaded sequence)	
Strain0014 (as downloaded sequence)	
Strain0015 (as downloaded sequence)	
Strain0016 (as downloaded sequence)	
Strain0017 (as downloaded sequence)	
Strain0018 (as downloaded sequence)	
Strain0019 (as downloaded sequence)	
Strain0020 (as downloaded sequence)	

86. Additional questions to the WGS part

87. Where was the sequencing performed

- (1) 🗆 In own laboratory
- (2) 🗆 Externally

88. Protocol used to prepare the library for sequencing:

- (1) \Box Commercial kits Go to 89
- (2) \Box Non-commercial kits Go to 91

89. Please indicate name of commercial kit:

90. If relevant please list deviation from commercial kit shortly in few bullets:

91. For non-commercial kit please indicate a short summary of the protocol:

92. The sequencing platform used

- (1) □ Ion Torrent PGM Go to 94
 (2) □ Ion Torrent Proton Go to 94
 (3) □ Ion S5 XL System Go to 94
 (4) □ Ion Genestudio S5 system Go to 94
 (5) □ Genome Sequencer Junior System (454) Go to 94
 (6) □ Genome Sequencer FLX System (454) Go to 94
 (7) □ Genome Sequencer FLX+ System (454) Go to 94
 (8) □ PacBio RS II Go to 94
 (9) □ PacBio RS Go to 94
 (10) □ HiScanSQ Go to 94
 (11) □ HiSeq 1000 Go to 94
 (12) □ HiSeq 1500 Go to 94
 (13) □ HiSeq 2500 Go to 94
 (14) □ HiSeq 2500 Go to 94
 (15) □ HiSeq 4000 Go to 94
- (16) \Box Genome Analyzer lix Go to 94
- (17) 🗆 MiSeq Go to 94
- (18) \Box MiSeq Dx Go to 94
- (19) 🗆 MiSeq FGx Go to 94
- (20) \Box ABI SOLiD Go to 94
- (21) 🗆 NextSeq Go to 94
- (22) 🗆 MinION (ONT) Go to 94
- (23) 🗆 Mini Seq Illumina Go to 94
- (24) \Box Other Go to 93

93. If another platform is used please list here:

94. Criteria used to evaluate the quality of sequence data.

In this section you can report criteria used to evaluate the quality of sequence data. Please first reply on the use of 5 selected criteria, which were the most frequently reported by in previous EQAs.

Next you will be asked to report 5 **additional** criteria of your own choice. For each criteria please also report the threshold or procedure used to evaluated the current criteria.

95. Did you use confirmation of species to evaluate the quality of sequence data?

- (1) 🗆 Yes
- (2) 🗆 No Go to 97

96. Procedure used to evaluate confirmation of genus:

97. Did you use coverage to evaluate the quality of sequence data?

- (1) 🗆 Yes
- (2) 🗆 No Go to 99

98. Procedure or threshold used for coverage:

99. Did you use Q score (Phred) to evaluate quality of sequence data?

- (1) 🗆 Yes
- (2) 🗆 No Go to 101

100. Threshold or procedure used to evaluate Q score (Phred):

101. Did you use genome size to evaluate the quality of sequence data?

(1) 🗆 Yes

(2) 🗆 No – Go to 103

102. Procedure or threshold used for genome size:

103. Did you evaluate the number of good cgMLST loci?

(1) 🗆 Yes

(2) 🗆 No – Go to 105

104. Threshold or procedure used to evaluate the number of good cgMLST loci:

105. ONLY list additional information related to other criteria used to evaluate the quality of sequence data.

Please list up to 5 additional criteria (e.g. N50, read length, contamination)

106. Other criteria used to evaluate the quality of sequence data - additional criteria 1:

107. Threshold or procedure used to evaluate the additional criteria 1:

108. Other criteria used to evaluate the quality of sequence data - additional criteria 2:

109.Threshold or procedure used to evaluate the additional criteria **2**:

110. Other criteria used to evaluate the quality of sequence data - additional criteria 3:

111. Threshold or procedure used to evaluate the additional criteria **3**:

112. Other criteria used to evaluate the quality of sequence data - additional criteria 4:

113. Threshold or procedure used to evaluate the additional criteria 4:

114. Other criteria used to evaluate the quality of sequence data - additional criteria 5:

115. Threshold or procedure used to evaluate the additional criteria 5:

116. Comment(s):

e.g. remarks to the submission, the data analyses or the laboratory methods

117. Please remember to upload your raw reads to the sFPT site:

https://sit-ftp.statens-it.dk/

Code: EQA_STEC13_upload

Have you remebered to upload your raw reads?

(1) O Yes

118. You have reached the end of the reporting scheme.

Please note that when you select "Yes" and "Next", your results will be automatically submitted and the reporting form will be locked.

If you wish to change your answers, use "Previous" to navigate backwards.

Upon completion, you will receive a link with your answers.

(1) 🗆 Yes

Thank you for your participation

Thank you for filling out the Submission form for the STEC EQA-13.

For questions, please contact ecoli.eqa@ssi.dk or phone +45 3268 8341

Remember to press "Finish" to complete submission.

After submission you will recieve a confirmation email with a link to the answers. We highly recommend to save this email.

Important: After pressing "Finish" you will no longer be able to edit or print your information.