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A comparison of three anti-HEV IgG EIA screening kits and one confirmatory immunodot assay in blood donor samples in Switzerland

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INTRODUCTION

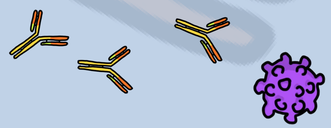
- Reported hepatitis E virus (HEV) seroprevalence varies widely between industrialised countries.
- Gold standard diagnostic algorithms are missing.
- We explored the sensitivity and specificity of three different enzyme immunoassay (EIA) kits in blood donor samples.

Country of study	Seroprevalence (%)	Year of publication	Subjects studied (n)	Laboratory test used
Italy	**1**	1994	948	Abbott
Netherlands	**1.1**	1993	1275	Abbott, Diagnostics Biotechnology
Switzerland	**3.2**	1994	94	Abbott, confirmation by Western-blot assay
N France	**3.2**	2007	1998	Genelabs Diagnostics
Switzerland	**4.9**	2010	550	MP Diagnostics, formerly Genelabs Diagnostics
England	**16**	2008	500	Wantai
SW France	**17**	2008	529	Genelabs Diagnostics
USA	**18**	2002	400	In-house
Denmark	**21**	2008	461	In-house

Table 1: Reported HEV seroprevalence in the literature

METHODS

- We collected 550 anonymised blood donor samples in the region of Lausanne, Switzerland. All samples were tested for the presence of anti-HEV IgG using three EIA screening kits:
  - **MP Diagnostics**
  - **Dia.Pro**
  - **Fortress**
- Any sample with an initial optical density (OD)/cut off ratio of ≥ 0.9 was retested in duplicate and was considered positive if the OD/cut off ratio of both replicates was ≥ 1.0
- Samples testing positive with ≥1 kit underwent confirmatory testing by an **immunodot assay** which is based on genotypes 1 and 3 :
  - **Mikrogen Diagnostik *recom*Line HEV IgG/IgM**
- Bands corresponding to one polypeptide are rated according to reaction strength and assigned a number of points. Samples are considered positive when reaching ≥4 points, borderline with 3 points and negative with ≤2 points.
- In order to calculate HEV seroprevalence we assumed two postulates:
  1) No positive samples test negative with all three EIAs and
  2) The *recom*Line immunodot is a reliable confirmatory test



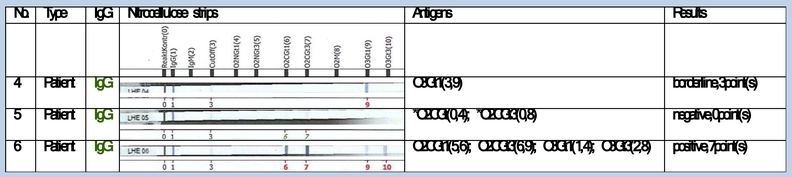


Figure 1: Scanned *recom*Line nitrocellulose strip showing borderline, negative and positive samples.

RESULTS

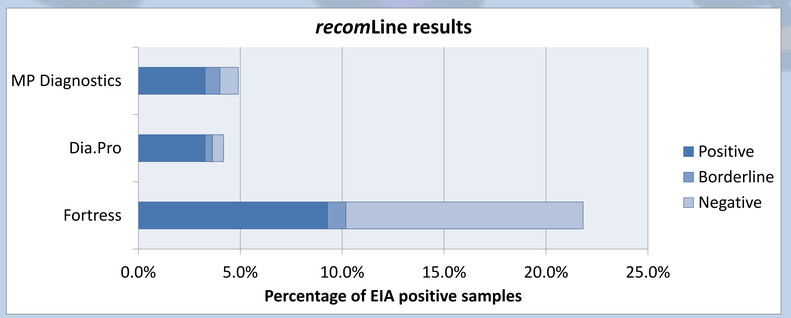
- 124/550 samples were positive with ≥1 EIA kit
- Depending on the combination of tests (one EIA ± confirmatory immunodot), seroprevalence varied from 3.3% to 21.8%.
- Taking only positive EIA samples confirmed with *recom*Line immunodot as positive tests, seroprevalences were 3.3% using the MP Diagnostics and Dia.Pro kits, and 9.3% using the Fortress kit.
- MP Diagnostics and Dia.Pro kits had identical sensitivity (35.3%) and similar specificity (98.2% and 99% respectively).
- The Fortress kit had higher sensitivity (100%) and lower specificity (86.2%).

EIA test result combinations (+, positive; -, negative)				Results with *recom*Line		
MP Diagnostics	Dia.Pro	Fortress	Total for each combination	Negative	Borderline	Positive
+	+	+	19	1	2	16
+	-	+	4	1	1	2
-	+	+	3	1	0	2
+	+	-	1	1	0	0
-	-	+	94	61	2	31
+	-	-	3	2	1	0
-	+	-	0	0	0	0
		Total	124	67	6	51

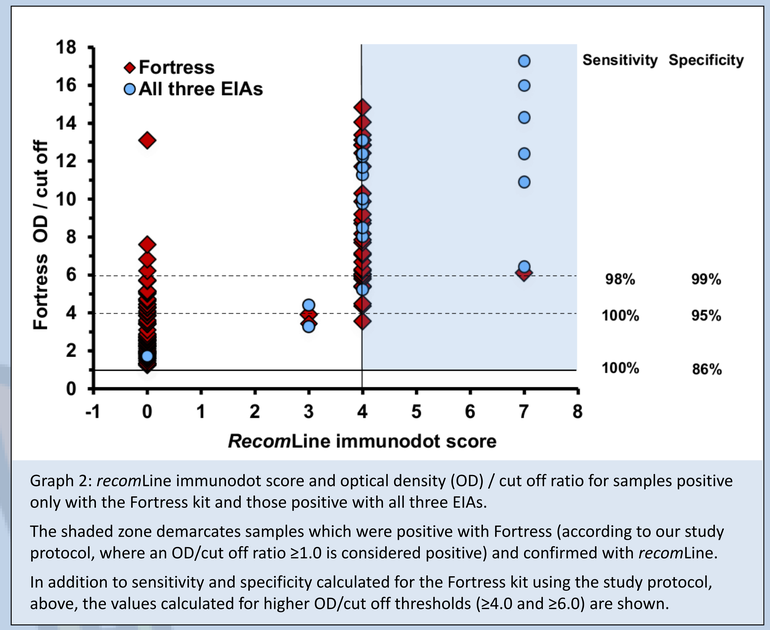
Table 2: *recom*Line results for each combination of EIA results

		Stringent criteria 'negative' *recom*Line = negative and BL samples (n=499); 'positive' *recom*Line = positive samples only (n=51)					Less stringent criteria 'negative' *recom*Line = negative samples only (n=493) 'positive' *recom*Line = positive and BL samples (n=57)				
	EIA test	*recom* Line -	*recom* Line +	Sensitivity	Specificity	Sero-prevalence	*recom* Line -	*recom* Line +	Sensitivity	Specificity	Sero-prevalence
MP Diagnostics	-	490	33	35.3	98.2	3.3	488	35	38.6	99	4
	+	9	18				5	22			
Dia.Pro	-	494	33	35.3	99	3.3	490	37	35.1	99.4	3.6
	+	5	18				3	20			
Fortress	-	430	0	100	86.2	9.3	429	1	98.2	87	10.2
	+	69	51				64	56			

Table 3: Sensitivities, specificities and seroprevalences calculated taking the *recom*Line immunodot as a gold standard confirmatory test and assuming that no truly positive samples were negative with all three EIAs.



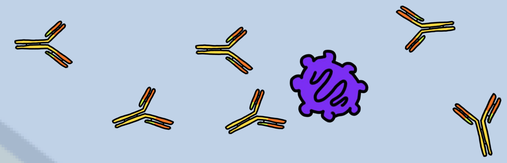
Graph 1: Prevalences obtained with each EIA and the corresponding *recom*Line result



Graph 2: *recom*Line immunodot score and optical density (OD) / cut off ratio for samples positive only with the Fortress kit and those positive with all three EIAs.

The shaded zone demarcates samples which were positive with Fortress (according to our study protocol, where an OD/cut off ratio ≥1.0 is considered positive) and confirmed with *recom*Line.

In addition to sensitivity and specificity calculated for the Fortress kit using the study protocol, above, the values calculated for higher OD/cut off thresholds (≥4.0 and ≥6.0) are shown.



CONCLUSION

- Taking our assumptions regarding the reliability of the *recom*Line immunodot and the absence of falsely negative EIA results as correct, we estimate HEV seroprevalence in this population to be close to 10%, twice that previously reported for this region.
- The Fortress EIA seems to lack specificity and may overestimate seroprevalence
- Seroprevalence reports using different tests should not be compared
- Further studies are required to evaluate *recom*Line as a gold standard confirmatory test.

REFERENCES

Kaufmann A, Kenfak-Foguena A, André C, Canellini G, Bürgisser P, Moradpour D, Darling KE, Cavassini M. Hepatitis E virus seroprevalence among blood donors in southwest Switzerland. PLoS One 2011;6(6):e21150. Epub 2011 Jun 20.