Diagnosis of suspected gastroenteritis patients using ELISA data, bacterial culture testing, API codes and NAAT testing

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Abstract:

This lab report presents the results of an investigation focused on the identification of bacterial pathogens into 8 patients, a series of diagnostic testing was done to attain diagnosis for each of the patients. The following diagnostic tests were used:
ELISA
Streak plate culture
API test strip
NAAT test
The first test conducted was Enzyme-Linked Immunosorbent Assay (ELISA) in which blood samples were tested for HIV antibody concentration, standard curve data was generated and compared to the patient data, patients with a threshold of >0.25ng/ml were identified as HIV positive. The clinical samples were then subject to bacterial streak plate culture testing to determine if a bacterial culture was present, followed by API 20E strip testing in order to generate API codes correlating to a database that identified the bacteria present. Finally a NAAT test was conducted using patient stool samples to identify if the patients were positive for STEC.

Introduction:

In the realm of infection science accurate identification of bacterial pathogens plays a vital role in patient treatment as well as disease control. Eight patients had been admitted to hospital with fever, vomiting and diarrhoea. The patients' symptoms are associated with suspected gastroenteritis. All eight patients are between the age of 18-68, with no history of recent travel. Stool and blood samples were taken from all eight patients and diagnostic tests were ordered. This report aims to present an evaluation of bacterial identification through MacConkeys agar streak plate testing, API 20E test strips and NAAT testing for STEC as well as ELISA testing for further insight into HIV status of the 8 patients.

Enzyme-Linked Immunosorbent Assay (ELISA) testing is a widely used, reliable form of testing mainly used to diagnose HIV in patients. In this report we used data from the ELISA test as well as standard curve data to generate a standard curve graph. We used the y= equation from the graph and applied it to our patient data, given the HIV antibody threshold of >0.25ng/ml we were able to give an acurate HIV status for each of our patients.

MacConkeys agar streak plate testing is a fundemental technique allowing us to isolate and cultivate bacterial colonies from clinical specemins and is an important step in the diagnosis of bacterial pathogens. Streak plate testing paired with Analytical Profile Index (API) testing and code analysis is a standardized yet reliable approach in not only determinanting if bacteria is the cause of illness but even identifying the specific bacteria present.

Last but not least we used NAAT testing as a sensitive molecular diagnostic tool to detect bacterial nucleic acids. This was done to determine if Shiga toxin-producing E. coli (STEC) was present in the clinical samples.

All methods are widely used and reliable forms of testing that ensure highly specific pathogen identification. They were performed with meticilous care, ensuring protocal and quality control methods were followed in order to produce accurate results and diagnosis. These molecular and biochemical techniques were used in aid of eachother in order to generate results and diagnosis.

Methods:

ELISA test for HIV status:

Step 1:

- Half of a 96-well micro-ELISA plate coated with TBE anti-gens in a bicarbonate buffer and left at room temperature for 2 hours, the other half that has not been coated with anything is marked and should not be used. During the experiment.
- Plate is inverted over a sink and a paper towel is used to remove the liquid
- The plate is immersed in phosphate-buffered saline (PBS)- tween, then inverted and vigorously tapped off over the sink, this is done to remove any unbound TBE antigens
- This is then repeated x2
- A blocking solution consisting of 1% skimmed milk powder mixed with PBS is then used to block any remaining protein binding sites.
- The plate is immersed in this mixture, with the wells completely full, ensuring there are no air bubbles, the plate is then incubated for 30 minutes at room temperature.
- After incubation the plate is then washed 3 times with PBS-tween, as described in previous steps

Step 2:

Using the following equipment -

Vial containing top standard (S6) for standard curve (1ng/ml of purified human anti-TBE lgG)

Vial containing dilatation buffer

6 Eppendorf tubes to prepare standard dilutions labelled S0-S5

8 Eppendorf tubes labelled P1-P8 which contain the patient serum samples to be tested

- micropipette used to dispense 350 µl of dilution buffer into tubes S0-S5
- pipette 350 µl from top standard tube S6 into S5 and mixed using vortex mixer
- 350 µl pipetted from S5 to S4 and mixed with vortex mixer
- repeat steps to prepare S3,2,1
- do not add S1 to S0 as S0 is blank

Concentrations of 2-fold standard dilutions:

Step 3:

100µl per well of standard (S) or test sample (P) is pipetted following this scheme:

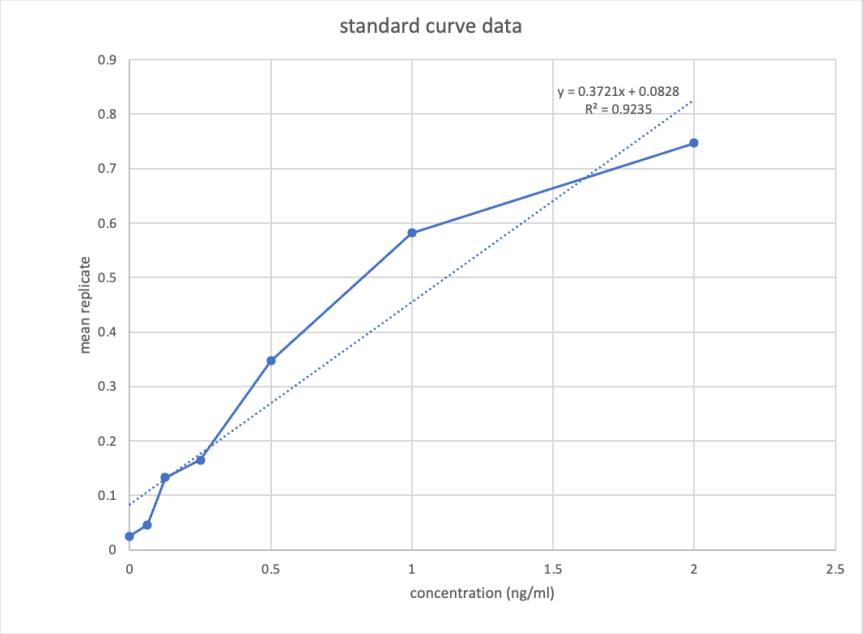
- Plate was then incubated for 30 minutes at room temperature
- After incubation plate was then washed 3 times with PBS-tween as previously described
- 100 µl of enzyme-conjugated anti-human lgG is pipetted into each well
- Incubated for 15 mins at room temperature
- Then rinsed 3 times with PBS-tween

- 100µl of TMB substrate was added to each well and it was left at room temperature for 5 minutes
- 50µl of 1M hydrochloric acid was added to each well using multichannel pipette in order to stop the reaction
- Absorbance measured at 450nm using ELISA plate reader

Standard curve data:

Concentration of HIV antibodies (ng/ml)	Replicate 1 (ng/ml)	Replicate 2 (ng/ml)	Replicate 3 (ng/ml)	Mean absorbance (ng/ml)
0	0.024	0.022	0.029	0.025
0.0625	0.049	0.044	0.044	0.046
0.125	0.133	0.132	0.135	0.133
0.25	0.164	0.163	0.167	0.165
0.5	0.344	0.342	0.356	0.347
1	0.579	0.584	0.583	0.582
2	0.741	0.744	0.756	0.747

Standard curve graph:



Patient data:

patient	replicate 1 (ng/ml)	replicate 2 (ng/ml)	replicate 3 (ng/ml)	average concentration (ng/ml)
1	0.024	0.039	0.033	0
2	0.114	0.177	0.133	0.0844
3	0.287	0.288	0.289	0.2264
4	0.198	0.194	0.192	0.1475
5	0.211	0.212	0.195	0.1588
6	0.073	0.074	0.073	0

7	0.122	0.121	0.123	0.0914
8	0.341	0.333	0.343	0.6519

Results:
Given that the HIV threshold is >0.25ng/ml these findings would suggest that patient 8 is HIV positive.

Bacterial culture and API generated codes:

Bacterial culture using MacConkey agar:
Method:

1) Label 8 MacConkey agar plates with patient number, date and time
2) Ensure the plates are at room temperature before use
3) Take the blood sample for patient 1 and thoroughly mix to ensure equal distribution of microorganisms.
4) Use a sterile cotton swab to streak the first quadrant of the plate back and forth horizontally
5) Take the inoculating loop and flame it to sterilize
6) Gently drag the inoculating loop through the first quadrant into the second to spread the bacteria
7) Flame the inoculating loop again and repeat to spread bacteria fron the second quadrant to the third
8) Flame the inoculating loop again and repeat to spread bacteria from the third to the fourth quadrant
9) Close and seal the MacConkey agar plate to prevent contamination
10) Incubate the plate in an incubator set to 37°C for 24 hours to allow for bacterial growth
11) Repeat for patients 2-8

Patient data	Bacterial culture
1	+
2	+
3	-
4	-
5	-
6	+
7	+
8	+

API 20E test strip:

Method:

1) Use the MacConkey agar plate already prepared
2) Steralise the inoculating loop
3) Pick up several well isolated bacterial colonies from the plate for patient 1
4) Add water to create a smooth bacterial suspension
5) Take an API 20E test strip and add a small sample of the bacterial solution to each of the tubes
6) Incubate the test strip at 37°C for 24 hours

7) After 24 hours remover from incubation and examine for any colour change
8) Use the chart provided to identify if each individual tube has a positive or negative result
9) Repeat for patients 2-8
10) Put the results into a chart in order to generate API code in order to identify what bacteria is present

API results:

	glu	fru	mal	sac	odc	ure	lip	pal	βgal	proA	ggt	ind	vp	gel	glu	man	ino	sor	rha	sac	mel	amy	ara
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	+	+	-	+
3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	+	+	-	+
7	-	-	+	+	-	+	-	-	-	-	-	+	+	-	+	+	-	+	-	+	-	-	+
8	+	-	+	+	-	-	-	-	+	-	-	+	+	-	+	-	+	+	-	+	+	-	+

API bacterial identification codes:

code	taxa
0044552	Escherichia coli
1044572	Escherichia coli
4144572	Escherichia coli
4304555	Salmonella spp.
5044552	Escherichia coli
5144552	Escherichia coli
5144562	Escherichia coli
5144572	Escherichia coli
6704752	Salmonella spp.

Bacterial identity based on API:

patient	Taxa based on API codes
1	Unable to determine
2	Salmonella spp.
3	n/a
4	n/a
5	n/a

6	Escherichia coli
7	Escherichia coli
8	Escherichia coli

Molecular based NAAT test for STEC:

Method:

1) Collect patient 1 stool sample
2) Place sample in a tube containing universal transport medium
3) Label the sample
4) Using NAAT test kit extract the DNA using extraction reagents
5) Run PCR using extracted genetic material
6) Compare results with positive and negative results to determine STEC presence
7) Repeat for patients 2-8

patient	results
1	-
2	+
3	-
4	-
5	-
6	+
7	-
8	+

Results

Patient 1: positive bacterial culture however no API code match found
Patient 2: API code suggests salmonella, positive for STEC
Patient 3: negative bacterial culture, negative STEC
Patient 4: negative bacterial culture, negative STEC
Patient 5: negative bacterial culture, negative STEC
Patient 6: API code suggests Escherichia coli, postitive for STEC
Patient 7: API code suggests Escherichia coli, negative STEC
Patient 8: API code suggests Escherichia coli, positive for both STEC and HIV

Discussion and conclusion:

The results obtained from our investigation using the diagnostic tests conducted gave us valuble insight into the status of each patient.
Patient 1: the bacterial culture test came back positive however no match was found when the API code was generated. No official diagnosis can be given and further testing into the

bacteria identity is required. This highlights a disadvanatge in the use of API testing as false negatives may have been given.
Patient 2: the bacterial culture test came back positive and the API test strip confirmed the presence of salmonella spp. Bacteria. The NAAT test also validated that the sample was positive for STEC bacteria. This highlights an advantage in using an API test strip in order to identify the bacterial pathogen
Patients 3,4,5: the initial bacterial culture test came back negative for any cultures meaning that the illness does not have a bacterial origin therefore further non-bacterial based testing is required for diagnosis.
Patient 6: API suggests escherichia coli is present, STEC was positive
Patient 7: API suggests escherichia coli however STEC was negative
Patient 8: API suggests escherichia coli, STEC was positive. Based of ELISA results the patient appears to be HIV positive.

In conclusion using these diagnositc tests allowed for a quick and cost-effective investigation to take place. These tests appear to be reliable and highly accurate given protocol is followed and that no possible contamination took place. However these diagnostic tests do have there disadvantages, all of the tests used are at risk of producting false negatives which is always a cause for concern therefore more advanced testing may be needed. It is also possible these tests can produce false positives due to thing like contamination, this once again puts emphasis on following correct lab protocol as well as carfeully undertaking procedures.

References:

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