MICROBIOLOGY EXTERNAL QUALITY ASSURANCE SCHEME
Under the aegis of Indian Association of Medical Microbiology
IAMM EQAS New Delhi
Department of Clinical Microbiology and Immunology
Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi-110060
NABL ISO/IEC 17043:2010 PC-1029

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Dated: 04-03-2020

Learning & Feedback on 22nd QC Package
Cycle 2019: Round 3 (November)

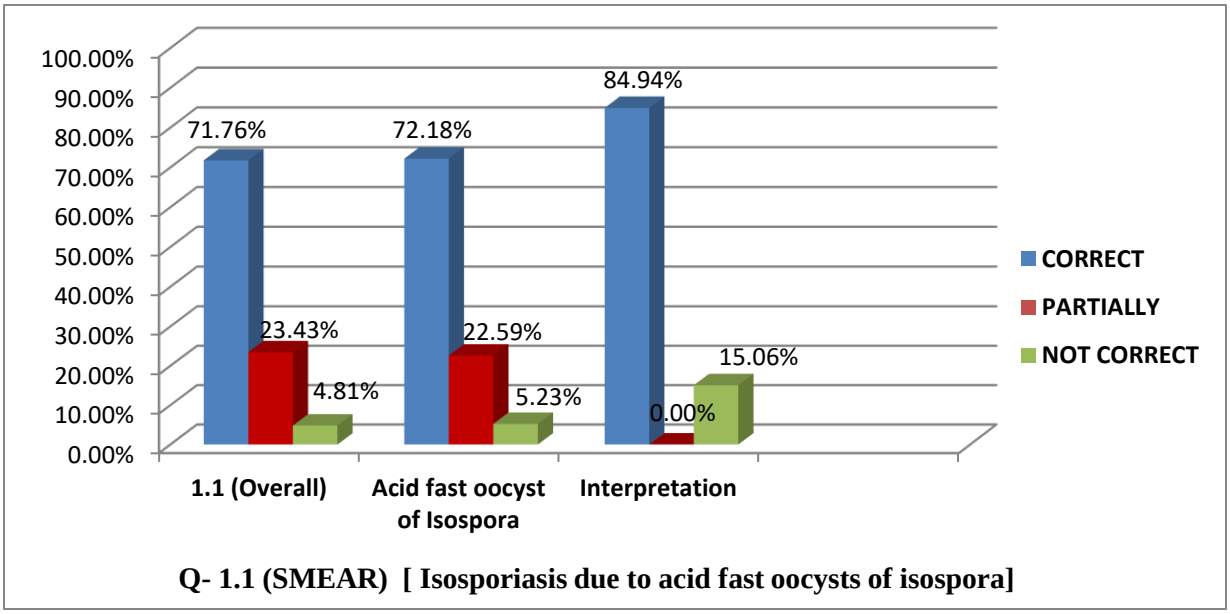
All samples were provided with necessary clinical details to help in interpretations that carry marks (cycle2019)

Smear examination: Using various stains

1.1 Stool sample for Kinyoun stain: 478 participants reported on this smear. Almost 85% of participants interpreted correctly the presence of *Isospora belli*. However, the overall correct reporting was done by approximately 72% of participants. 23% participants did not mention the acid fast character of oocysts, which is the most differentiating feature, therefore, have lost marks. Isosporiasis is not very uncommon and can easily be differentiated on the basis of its size and the diagnosis can be arrived at quickly in patients suffering from abdominal symptoms and diarrhea. Further details can be seen from figure 1. 15% participant diagnosed Isospora as cryptosporidium. The important differential for Isospora remains *Cryptosporidium parvum* & *Cyclospora spp*. The former is the smallest in size (3-5 µm), spherical in shape, infectious & the latter is 8-10 µm in size, spherical or slightly oval and infectious when passed in faeces. Whereas *Isospora belli* is 20-30 µm x 10-29 µm in size ellipsoidal in shape with 2 sporozoites inside & infectious when passed in this stage with stool.

RESULT ANALYSIS
22nd QC Package, Cycle 2019, Round 3(November)
Total participants in EQAS scheme (n=560)
SMEAR

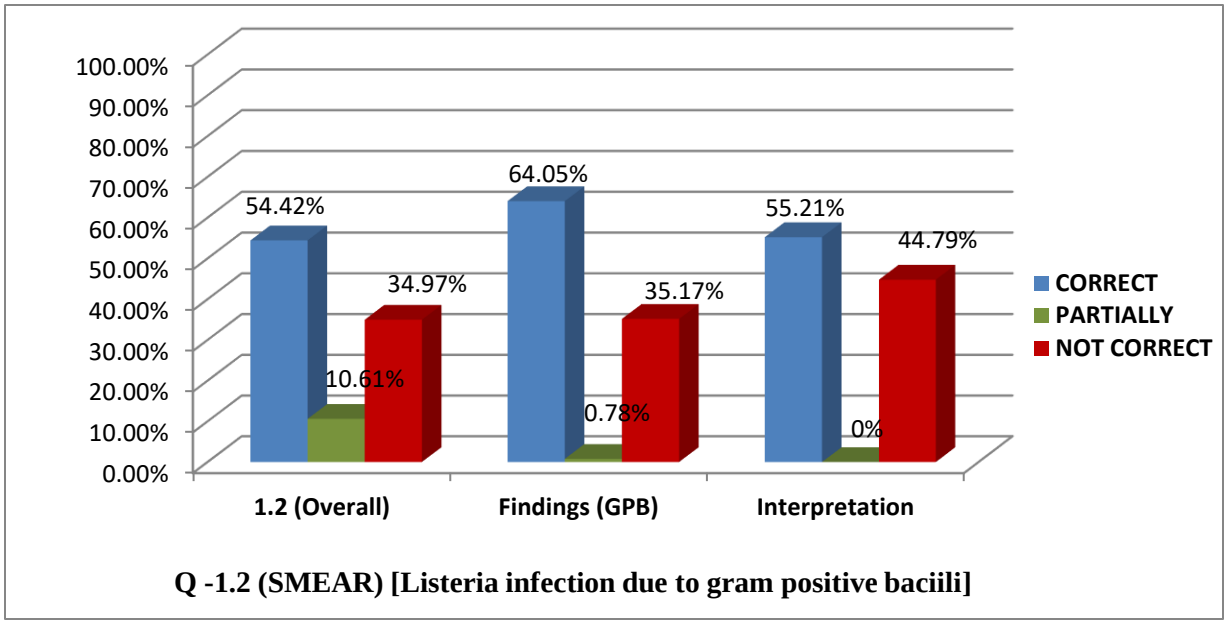
Fig 1: Stool Sample, Kinyoun Stain (n = 478)



Q- 1.1 (SMEAR) [Isosporiasis due to acid fast oocysts of isospora]

1.2 *Blood culture broth for gram stain: 509 participants reported on this smear. Only half of the participants identified the organism as *Listeria* correctly. 45% participants wrongly reported its gram reaction as gram negative bacilli resembling *Haemophilus influenzae* and few others also reported it as *Corynebacterium sps*. Over all 10% were partially correct and 40% to 45% faulted in giving the findings or the interpretations therefore, were not correct. Other results can be seen in figure 2.

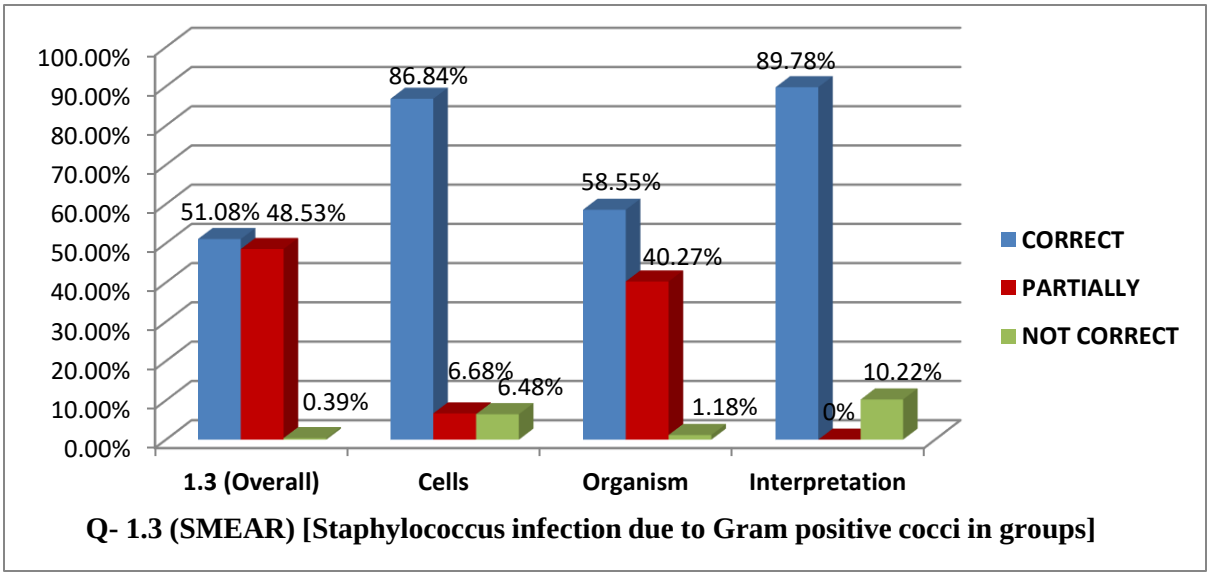
Fig 2: Blood Culture Broth, Gram Stain (n = 509)



Q -1.2 (SMEAR) [Listeria infection due to gram positive baciili]

1.3 Pus Sample for gram staining: 509 participants participated. *Staphylococcus* was identified by 89.78% participants. Marks were lost because half did not quantify. Surprisingly, only half of the participants applied their mind to semiquantify gram positive cocci in groups resembling *Staphylococci*, therefore, lost marks. Though, gram is not a good stain for identifying cellular reaction, however, 87% participants reported acute cellular reaction correctly. 58.5% participants identified gram positive cocci as *Staphylococci* correctly. Almost 90% participants interpreted correctly. (Fig. 3) To quantify grossly the amount of inflammatory cells present & the number of organisms gives additional confidence to the treating physician to consider the diagnosis of Staphylococcal abscess.

Fig 3: Pus Sample, Gram Stain (n = 509)



Q- 1.3 (SMEAR) [Staphylococcus infection due to Gram positive cocci in groups]

Q2: Culture & Sensitivity

2.1, 2.2 & 2.3 : Culture and Sensitivity:

498 participants participated in this exercise. Overall 68%, 70% & 69% participants conducted identification and ABST correctly Table 1 & Fig. 4. One fourth of participants were partially correct means a good scope of moving up to completely correct status.

Table 1: Culture Identification & Sensitivity (n=498)

QC No. 22	Description (Culture Identification & Sensitivity)	Lab (%) Giving No. Correct Result
(2.1)	*Shigella dysenteriae*	Correct- 68.48% Partially Correct- 26.10% Not Correct- 5.42%
(2.2)	*Enterococcus faecalis*	Correct- 69.68% Partially Correct- 28.71% Not Correct- 1.61%
(2.3)	*Acinetobacter baumannii complex*	Correct- 69.48% Partially Correct- 27.71% Not Correct- 2.81%

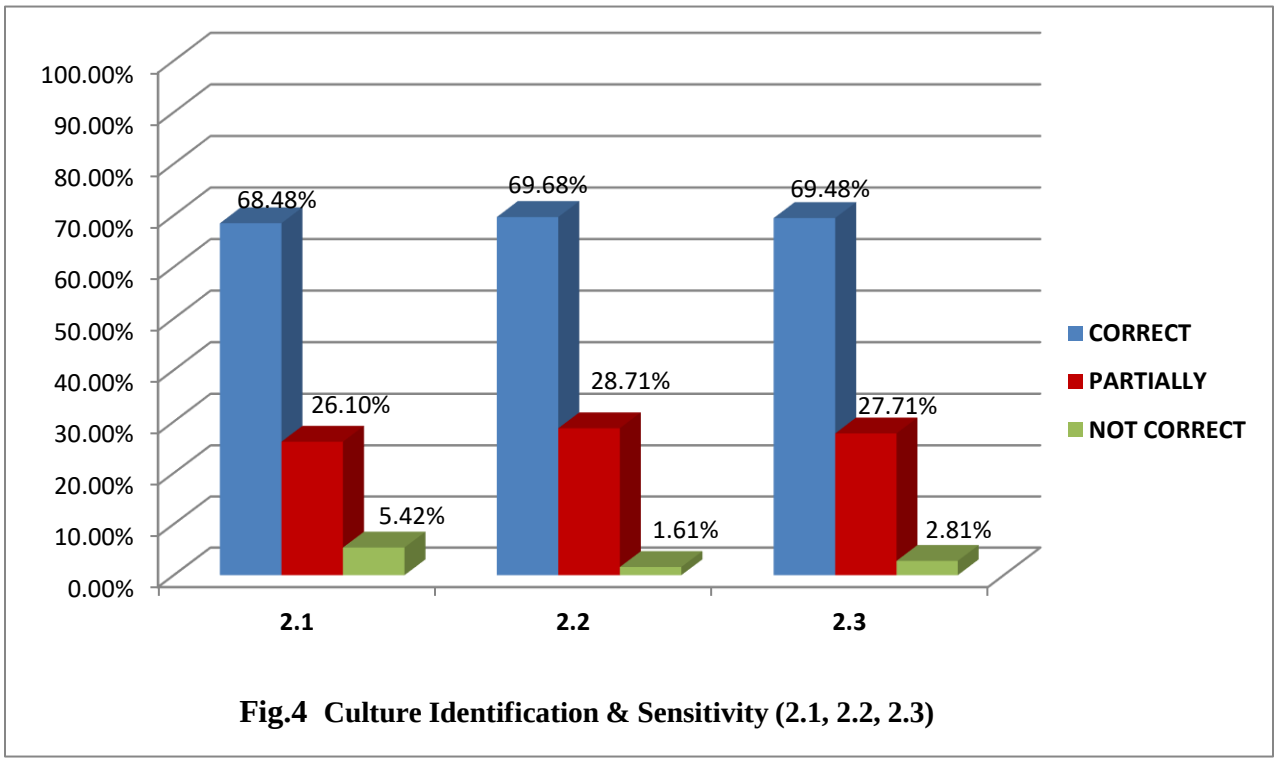


Fig.4 Culture Identification & Sensitivity (2.1, 2.2, 2.3)

Culture Identification: 90.1%, 96% and 93.5%; 98.25, 100 & 99.67% participants correctly identified *Shigella dysenteriae, Enterococcus faecalis* and *Acinetobacter baumannii* complex, using manual & automated systems respectively Table 2(a).

Table 2(a): Culture Identification
Various methods used by the participants (n=498)

Identification Method Details	2.1 *Shigella dysenteriae*	2.2 *Enterococcus faecalis*	2.3 *Acinetobacter baumannii complex*
Total Response (498)	**Correct Response/ Total Response (%)**		
1- Manual Method	**191/212 (90.09%)**	**192/200 (96%)**	**186/199(93.47%)**
2- Automated Method	**281/286 (98.25%)**	**298/298 (100%)**	**298/299 (99.67%)**
(i) VITEK-2	239/240 (99.58%)	248/248 (100%)	247/248 (99.60%)
(ii) Microscan	16/16 (100%)	17/17 (100%)	16/16 (100%)
(iii) BD Phoenix	24/25 (96%)	22/22 (100%)	24/24 (100%)
(iv) MALDITOF	0/2 (0%)	8/8 (100%)	8/8 (100%)
(v) DL-96	2/3 (66.67%)	3/3 (100%)	3/3 (100%)

Culture sensitivity: In ABST testing for *Shigella dysenteriae*, 193 and 274 performed ABST manually and using automated method, respectively. Vitek-2 was used by 99.58% participants. 4.25% participants had very major errors for Ciprofloxacin. Other details of the AST results can be seen in table 2 (b). When fecal isolates of *Salmonella* and *Shigella* spp. are tested, only ampicillin, a fluoroquinolone and trimethoprim-sulfamethoxazole should be reported routinely. For *Salmonella* spp. and *Shigella* spp., 1st- and 2nd-generation cephalosporins and cephamycins may appear active *in vitro* but are not effective clinically and should not be reported as susceptible. In *Enterococcus faecalis* unacceptable rate of categorical error (19.4%) was seen for penicillin testing by disc diffusion method. Penicillin was falsely reported as resistant. This could be due to the fact that certain beta lactam antibiotics like penicillin are very labile and prone to lose potency if not stored under proper environmental conditions of temperature and humidity. Therefore it is highly recommended that there should be very stringent quality control before using these antibiotic discs. Similarly high rate of categorical error (11.1%) was noted for vancomycin in automated methods. The automated systems need to undergo regular QC to review this error. There was no very major error committed by any laboratory in the ABST of *Enterococcus faecalis*. To read manual ABST hold the Petri plate a few inches above a black background illuminated with reflected light, except for vancomycin, which should be read with transmitted light (plate held up to light source). Any discernible growth within the zone of inhibition indicates vancomycin resistance. *Enterococcus* spp., aminoglycosides (except for high-level resistance testing), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole may appear active *in vitro*, but they are not effective clinically, and isolates should not be reported as susceptible. Synergy between ampicillin, penicillin, or vancomycin and an aminoglycoside can be predicted for enterococci by using a high-level aminoglycoside (gentamicin and streptomycin) test. The result of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Ampicillin results may be used to predict susceptibility to amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam among non-β-lactamase-producing enterococci. Enterococci susceptible to ampicillin cannot be assumed to be susceptible to pencillin. If penicillin results are needed, testing of penicillin is required.

The ABST of *Acinetobacter baumannii* was performed correctly almost by all the participants. 35 (7.2%) participants did not attempt colistin testing. 84 participants used disc diffusion for colistin AST and these results were not considered for the analysis of colistin AST as the interpretative criteria is not available for disc diffusion. 39 participants attempted colistin sensitivity using broth dilution which is considered as the reference method for colistin AST. 235, 53, 20 & 15 participants did colistin testing using Vitek-2, E-Test, BD Phoenix & Microscan, respectively. Inspite of the advisories from the manufacturer 99.27% participants could report

colistin sensitivity correctly by automated methods. Only 0.82% major errors were detected for colistin by all automated methods.

However, results can be seen from table 2 (b) and figures 5, 6 & 7. The participants who did not attempt any particular antibiotic testing were put in the category of having not performed test and the marks were not earned. All these participants had ABST in their scope, therefore, cannot be exempted from evaluation.

For colisin AST, apart from BMD, colistin broth disk elution (CBDE) and colistin agar test (CAT) are considered as acceptable methods by the new CLSI M 100, 30th Ed., 2020 document. Colistin and polymyxin B are considered equivalent agents, so MICs obtained from testing colistin predict MICS to polymyxin B and vice versa. CBDE and CAT are currently recommended for *enterobacterales* and *P.aeruginosa.* Colisitn epidemiological cutoff value (ECV) have been deleted and now assigned a breakpoint. The new interpretive MIC breakpoints are: $\leq$ 2 as intermediate and $\geq$ 4 as resistant. Susceptible category has not been assigned to colistin. Clinical and PK-PD data demonstrate colistin and polymyxin B have limited clinical efficacy, even if an intermediate result is obtained.

Table 2(b): Culture Sensitivity

Total Response 498	**Automated Method (MIC)**	**Manual Method (Disc Diffusion)**	**Not attempted n(%)**	**Very Major Error n(%)**	**Major Error n(%)**	**Minor Error n(%)**
(2.1) *Shigella dysenteriae* (n=471)*	**Correct Response/ Total Response (%)**	**Correct Response/ Total Response (%)**				
Ampicillin (R)	273/274 (99.63%)	187/193 (96.89%)	4 (0.85%)	6 (1.28%)	-	1 (0.21%)
Ceftriaxone (S)	259/264 (98.11%)	202/206 (98.06%)	1 (0.21%)	-	7 (1.49%)	2 (0.42)
Ciprofloxacin (R)	282/286 (98.60%)	150/184 (81.52%)	1 (0.21%)	20(4.25%)	-	18 (3.83%)
Meropenem (S)	284/285 (99.65%)	178/184 (96.74%)	2 (0.42%)	-	5 (1.07%)	2(0.43%)
Cefepime (S)	285/285 (100%)	170/180 (94.44%)	6 (1.27%)	-	4(0.86%)	6(1.29%)
***27 Participants wrongly identified the given organism.**						
(2.2) *Enterococcus faecalis* (n=490)*						
Gentamicin High Level (S)	279/281 (99.29%)	197/206 (95.63%)	3(0.61%)	-	8(1.64%)	3(0.62%)
Linezolid (S)	296/297 (99.66%)	188/190 (98.95%)	1(0.20%)	-	3 (0.61%)	2(0.41%)
Penicillin (S)	284/290 (97.93%)	154/191 (80.63%)	9(1.84%)	-	40 (8.32%)	3(0.62%)
Tetracycline (S)	288/289 (99.65%)	194/197(98.48%)	4(0.82%)	-	3(0.62%)	1 (0.21%)
Vancomycin (S)	265/298 (88.93%)	176/187(94.12%)	5(1.02%)	-	39(8.04%)	5(1.03%)
***08 Participants wrongly identified the given organism.**						
(2.3) *Acinetobacter baumannii complex* (n=484)*						
Amikacin (R)	239/241 (99.17%)	225/237 (94.94%)	6(1.24%)	13(2.72%)	-	1 (0.21%)
Ciprofloxacin (R)	294/295 (99.66%)	185/189 (97.88%)	-	3(0.62%)	-	2 (0.41%)

Colistin (S)	271/273 (99.27%)	** Disc diffusion- 0/84 E-Test- 53/53(100%) BMD- 38/39 (97.44%)	35 (7.23%)	-	3(0.82%)	-
Meropenem (R)	294/295 (99.66%)	167/186 (89.78%)	3 (0.62%)	16(3.33%)	-	4 (0.83%)
Piperacillin/ Tazobactam (R)	292/293 (99.66%)	185/190(97.37%)	1 (0.21%)	4 (0.83%)	-	2 (0.41%)

*** 14 Participants wrongly identified the given organism.**

**** Disc diffusion results were not considered for the analysis of colistin AST as the interpretive criteria is not available for disc diffusion.**

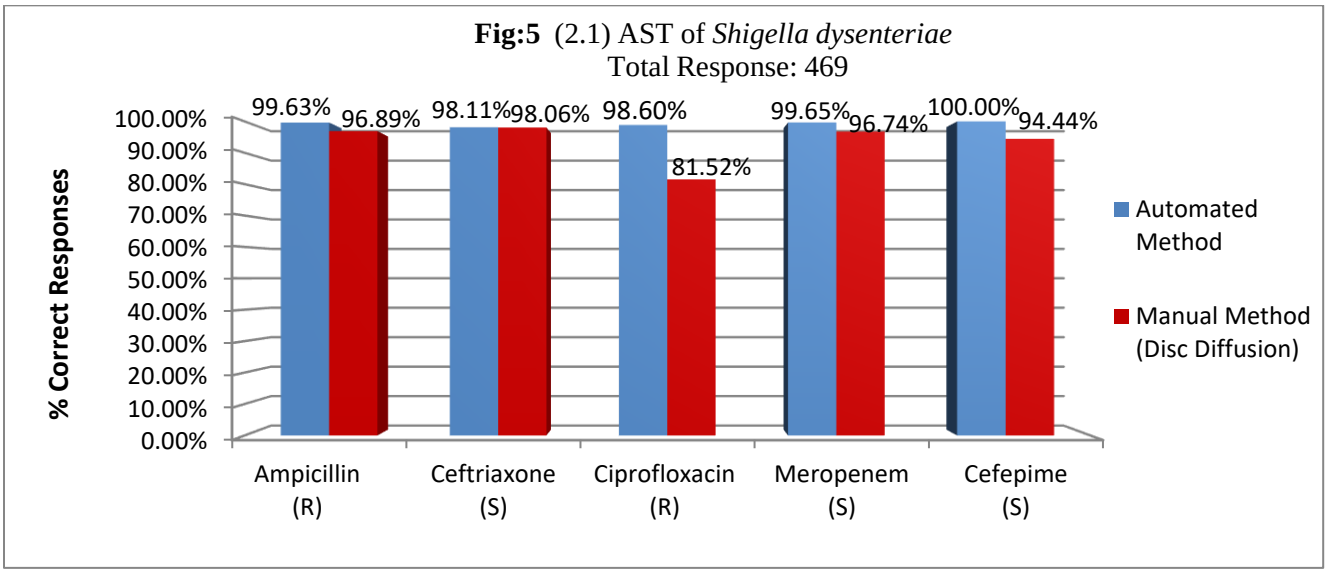


Fig:5 (2.1) AST of *Shigella dysenteriae*
Total Response: 469

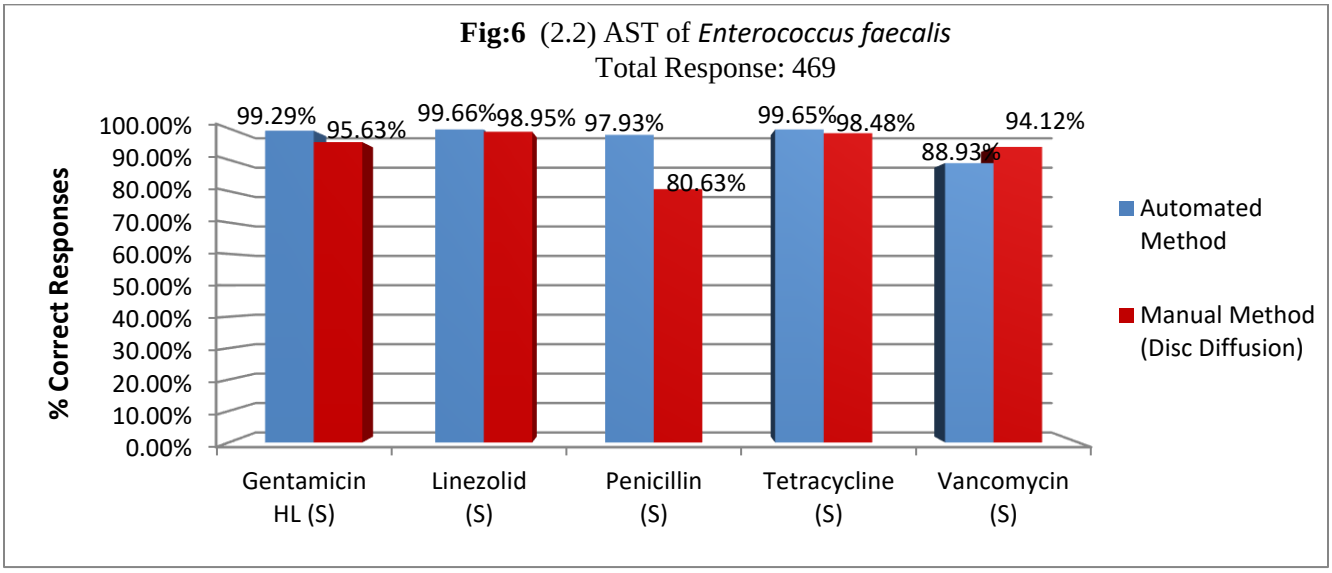
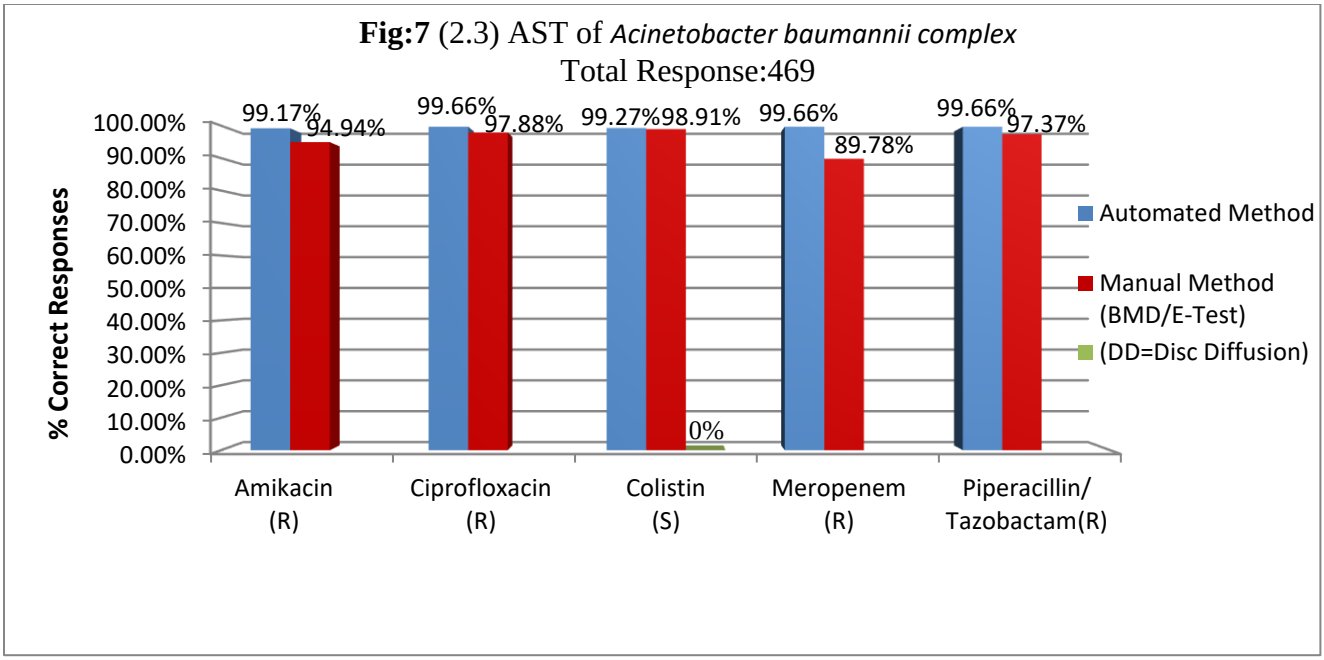


Fig:6 (2.2) AST of *Enterococcus faecalis*
Total Response: 469



Total Participants – 560

SEROLOGY & BBV

3.1.1 & 3.1.2

Out of 560 participants 438, 208 & 514 participated for typhidot (IgM), CMV-IgG serology and HIV, HBsAg, HCV for BBV, respectively. 96% participants reported typhidot (IgM) and CMV-IgG correctly whereas, almost 100% participants reported BBV correctly. The details can be seen from table 3 and figures 8 – 16. ELISA as a method for detecting IgM antibodies for *Salmonella typhi* was correctly detected by 89% participants whereas the other methods performed between 95 – 98%. Participants using card test for CMV-IgG positive performed sub optimally by being correct only 60% of the times whereas CLIA, ELISA and ELFA performed correctly more than 96% of the times.

All the participants have performed well. In the panel 3.2.1 the correct responses have varied between 98.15% to 100% using various assays. The break up is provided in the figures. Similarly, is 3.2.2 panel most of the participants have performed well with correct responses ranging between 91.67 to 100% except in HBsAg. Negative sera in the 3.2.2 panel surprisingly only 61.54% participants having used CMIA have got true negatives & the rest have reported false positive HBsAg. However, ELFA & CLIA have performed between 90-95% as compared to the 100% by other techniques. This again points towards the quality control of sero assays.

Table - 3

QC No. 22	Description Serology & BBV	Total Responses	Lab (%) Giving No. Correct Result
(3.1.1)	Typhidot (IgM)	438	Correct- 96.12% Not Correct- 3.88%
(3.1.2)	CMV- IgG	208	Correct- 96.15% Not Correct- 3.85%
(3.2.1)	HIV, HBsAg, HCV	514	Correct- 99.22% Partially Correct- 0.78%
(3.2.2)	HIV, HBsAg, HCV	514	Correct- 99.22% Partially Correct-0.78 %

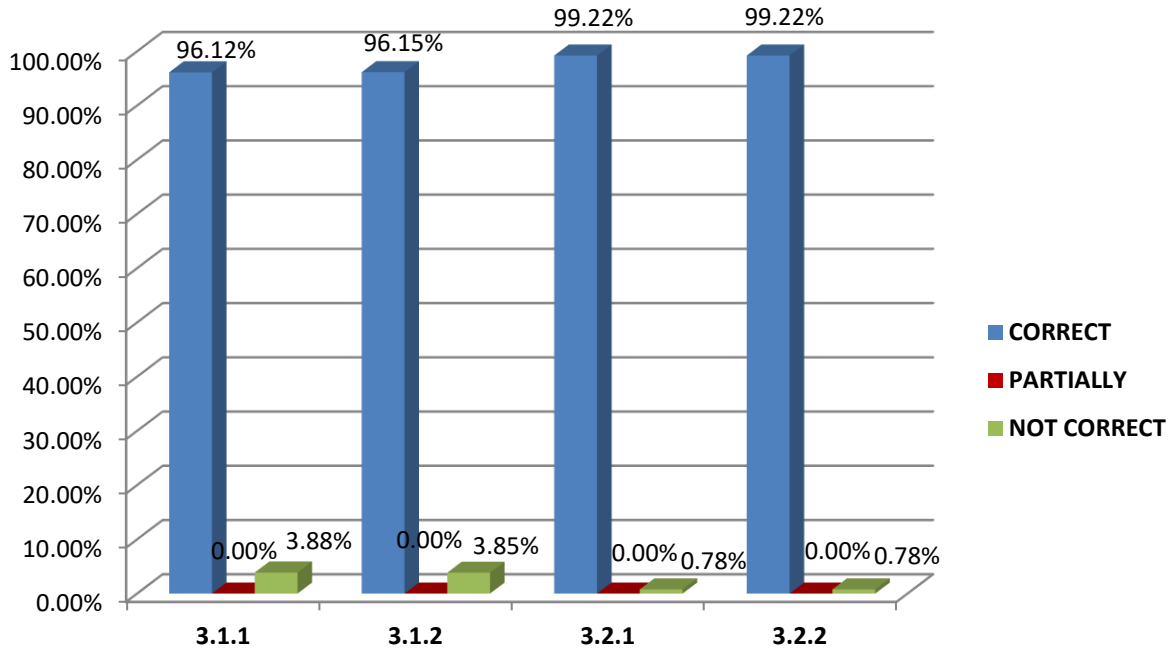
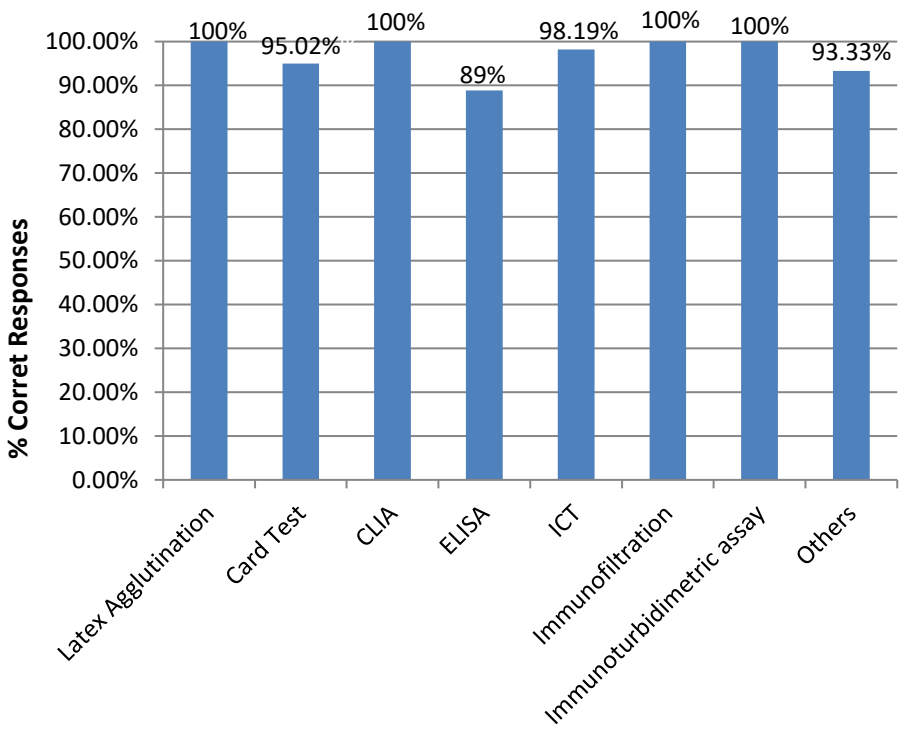


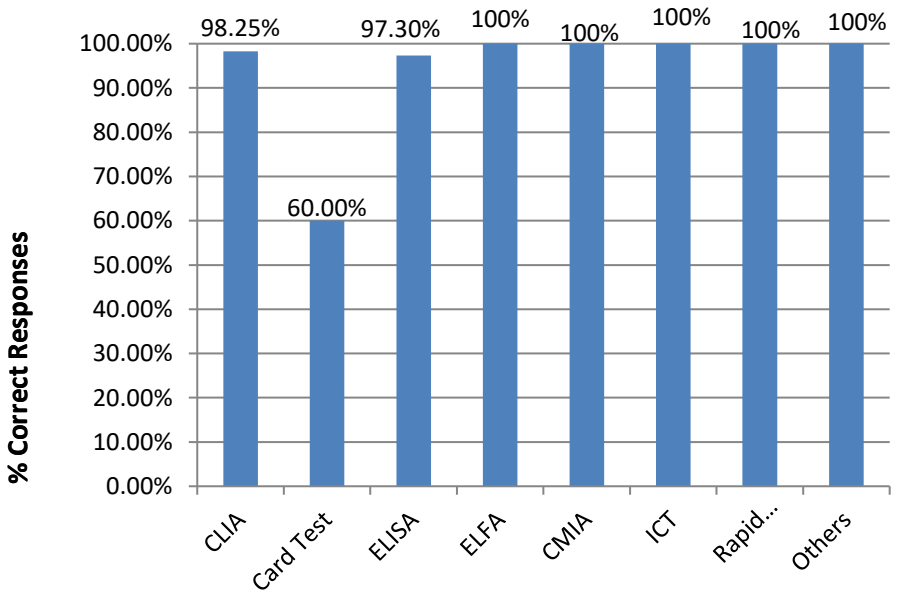
Fig:8 Serology & BBV (3.1.1, 3.1.2, 3.2.1 & 3.2.2)

Fig:9 (3.1.1) Typhidot IgM [Negaitive]



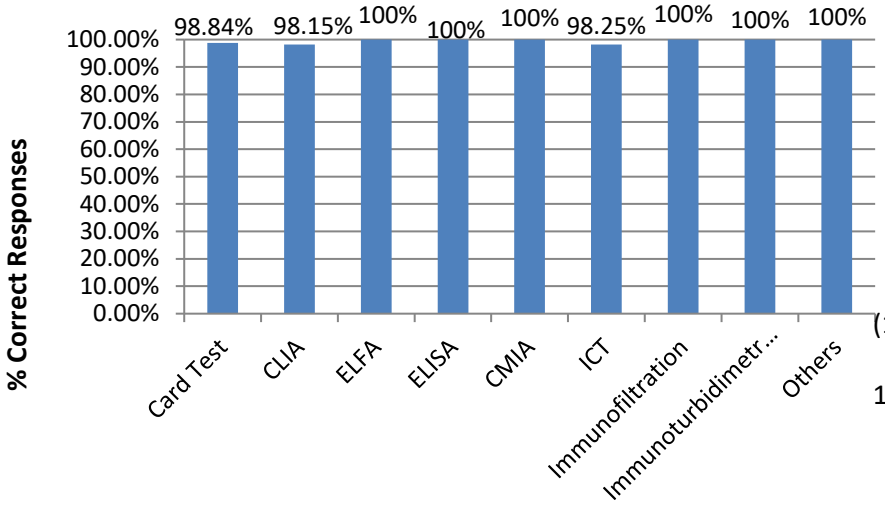
Total Response - 438
Method Used :
Latex Agglutination-1/1(100%)
Card Test - 229/241 (95.02%)
CLIA - 1/1 (100%)
ELISA -8/9 (88.89%)
ICI - 163/166 (98.19%)
Immunofiltration- 3/3(100%)
Immunoturbidimetric assay-2/2 (100%)
Others - 14/15 (93.33%)

Fig:10 (3.1.2) CMV- IgG [Positive]



Total Response - 208
Method Used :
CLIA-56/57 (98.25%)
Card Test-6/10 (60.00%)
ELISA - 72/74 (97.30%)
ELFA - 41/41 (100%)
CMIA-22/22 (100%)
ICT- 1/1 (100%)
Rapid Immuno-chromatography-1/1 (100%)
Others- 2/2 (100%)

Fig:11 (3.2.1) HIV [Negative]



Total Response - 513

Method Used :

Card Test-170/172(98.84%)

CLIA - 106/108(98.15%)

ELFA - 12/12(100%)

ELISA - 53/53(100%)

CMIA- 40/40(100%)

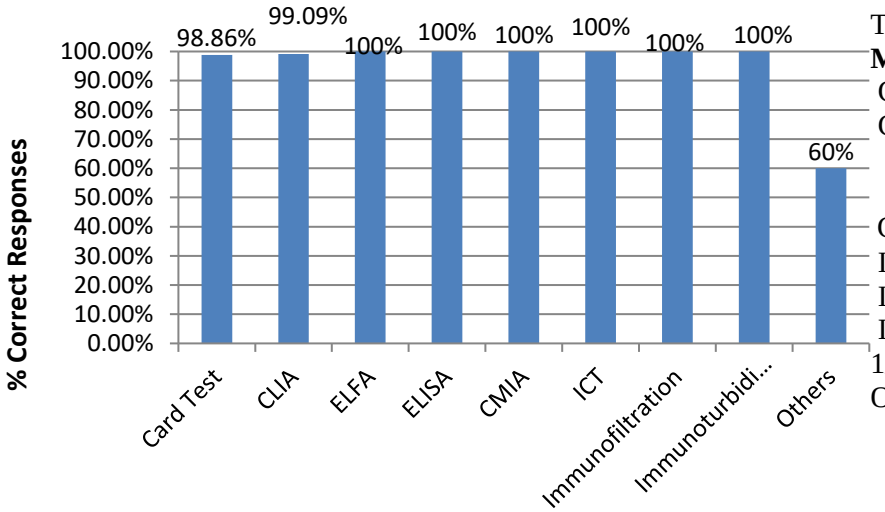
ICT- 56/57 (98.25%)

Immunofiltration -36/36 (100%)

Immunoturbidimetric assay- 1/1(100%)

Others - 35/35(100%)

Fig:12(3.2.1) HBsAg [Positive]



Total Response - 513

Method Used :

Card Test-174/176(98.86%)

CLIA -109/110(99.09%)

ELFA - 16/16(100%)

ELISA - 68/68(100%)

CMIA- 40/40(100%)

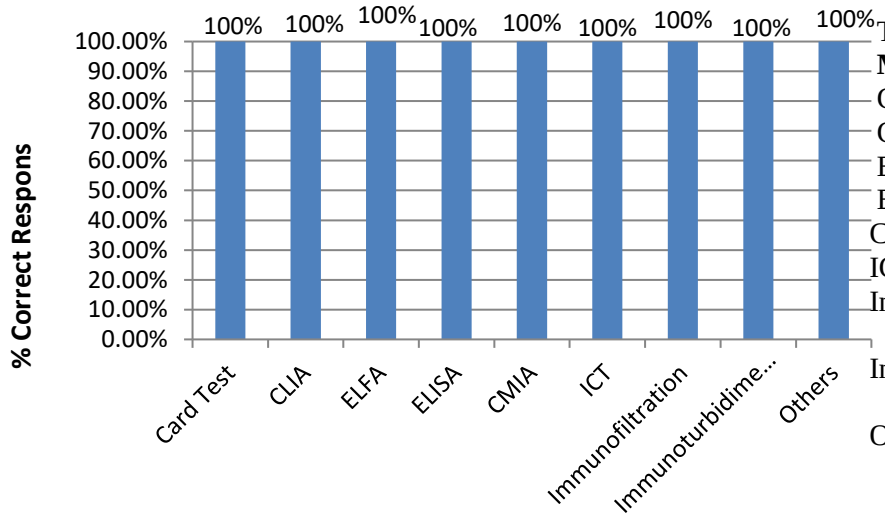
ICT -84/84(100%)

Immunofiltration-6/6(100%)

Immunoturbidimetric assay- 1/1(100%)

Others - 12/20(60%)

Fig:13(3.2.1) HCV [Negative]



Total Response - 511

Method Used :

Card Test-168/168(100%)

CLIA - 107/107(100%)

ELFA - 14/14(100%)

ELISA- 74/74(100%)

CMIA -40/40(100%)

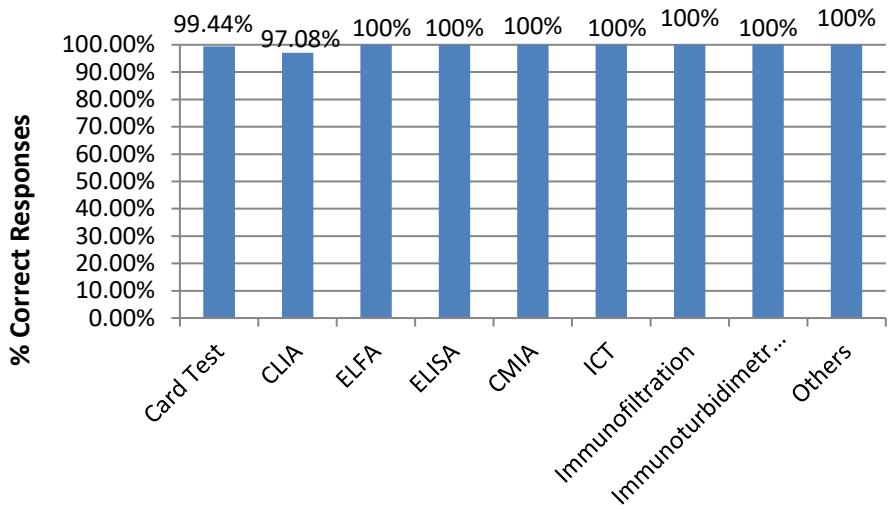
ICT -69/69(100%)

Immunofiltration- 28/28(100%)

Immunoturbidimetric assay - 1/1(100%)

Others - 11/11(100%)

Fig:14 (3.2.2) HIV [Positive]

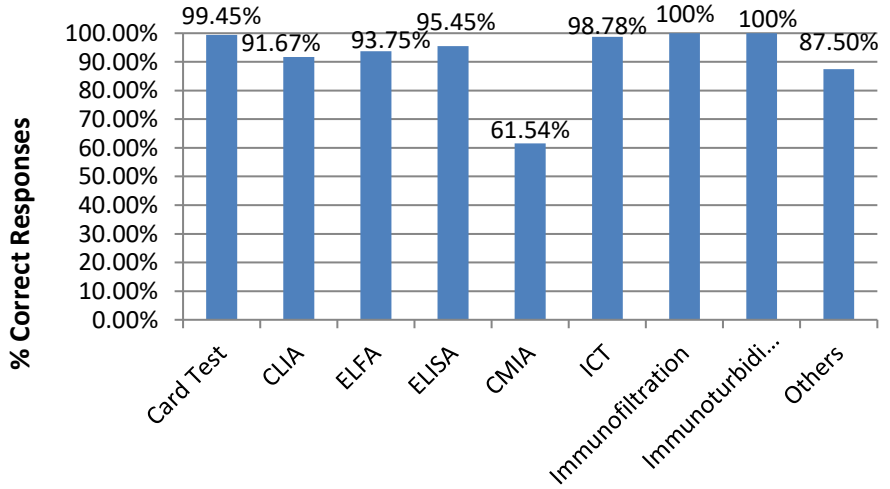


Total Response - 514

Method Used :

Card Test-178/179 (99.44%)
CLIA - 100/103(97.08%)
ELFA - 12/12(100%)
ELISA- 53/53(100%)
CMIA- 40/40(100%)
ICT - 56/56(100%)
Immunofiltration-36/36(100%)
Immunoturbidimetric assay- 1/1(100%)
Others - 34/34(100%)

Fig:15 (3.2.2) HBsAg [Negative]

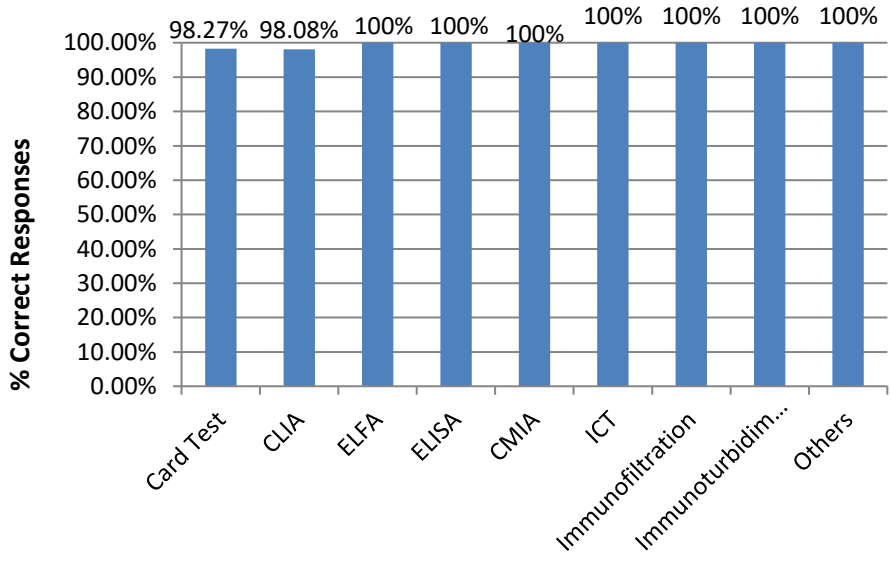


Total Response - 512

Method Used :

Card Test-182/183(99.45%)
CLIA -99/108(91.67%)
ELFA - 15/16(93.75%)
ELISA-63/66(95.45%)
CMIA -24/39 (61.54%)
ICT - 81/82(98.78%)
Immunofiltration-7/7(100%)
Immunoturbidimetric assay- 1/1(100%)
Others - 7/8(87.50%)

Fig:16(3.2.2) HCV [Negative]



Total Response - 511

Method Used :

Card Test-170/173 (98.27%)
CLIA-102/104 (98.08%)
ELFA - 14/14 (100%)
ELISA - 73/73 (100%)
CMIA- 40/40(100%)
ICT - 67/67(100%)
Immunofiltration- 29/29(100%)
Immunoturbidimetric assay- 1/1(100%)
Others - 10/10(100%)

General remarks & response to feedbacks

Dear Colleagues,

We have ended the year 2019, the 22 QC package of EQAS with 560 participants. Al the cycles of 2019 were paperless. Hope you all appreciated the usefulness of this version of our EQAS. However, glitches were expected and I thank you all for your patience in handling specific issues. I would encourage all participants to kindly use the EQAS contact nos. in case of difficulty without any hesitation. Our attempt has been always to be all inclusive rather than exclusive. Having said that, there have been various mails that have been received by us regarding their difficulties in understanding our evaluation system and has resulted in anxiety to some of our participants because of having lost some marks here or there. Kindly understand we need to take EQAS to the next level wherein the interpretation of medical microbiology is further strengthened. As a result only gram reaction or stain reaction is not the only expectation but the cellular and other morphological details are equally important in arriving at a diagnosis and correct interpretation. We tried hard to provide you with the moderate to high challenges to improve the overall quality of our participants in medical microbiology work. As and when the mails are received from the participants we try and respond. In case those are beyond the scope of the EQAS admin the reply to such mails are provided in this feedback evaluation. Therefore, wait for your answer in this learning and feedbacks to the QC package at the end of each cycle. Many of our participants have lost marks in colistin testing. Hope the above reflections on Colistin sensitivity in line with the current CLSI guidelines will satisfy your queries. Any participants who claims that he or she has not done colistin testing because of the absence of recommended method does not absolve the participating lab of performing the test in the era of multi drug resistance. In the interest of patient care, therefore, such reasons cannot be the reason of exclusion for evaluation. Many participants who have used disc diffusion as the method of colistin testing may realize that these findings are unreliable and the technique not recommended, therefore, cannot be evaluated and marks given. Kindly note that in smear examinations simply writing the name of organism and not providing the basic interpretative criteria cannot earn all the marks. As mentioned earlier the evaluation of smears is based on all investigative parameters and the participanting laboratory is also being prompted to provide various findings under various headings e.g. question number 1.3.

Kindly note that the following information was mailed to all participants but still some transactions are being made in the old account. The refund from such account is not now possible so kindly be mindful before the older account is used. This amount cannot be refunded:

The previous account of **"IAMM EQAS DELHI", Account No 9112010067240** has been closed and no payment will be accepted in this account. No transaction tracking will be possible. If amount is still transacted on the above henceforth closed account it will get returned by the bank which may take time for you to realize but by then you could loose a cycle or two. Kindly be mindful about this announcement.

The current account details are given below:
Account Beneficiary name: IAMM EQAS NEW DELHI TRUST
Account no: 91112010084978
IFSC code: SYNB0009111
Bank Name and Address: Syndicate Bank, Sir Ganga Ram Hospital, Rajinder Nagar,
Delhi- 110060

Only 23 participants have sent their feedback online as a result the findings are not interpretable. Kindly do the same ASAP. Your feedbacks are vital for our course correction and for constant improvement.

Note:

- The identity and results of the participants are kept confidential. Participants must consult the scheme organizer before quoting data from the scheme.
- Production of PT items: Smears are prepared from the clinical samples/culture isolates. Bacterial strains are obtained from clinical samples or ATCC strains. Pooled patients' sera or commercially bought freeze dried reagents are used for serological testing.
  IAMM EQAS NEW DELHI is a qualitative PT scheme, the assigned value of the PT Items for the 22nd QC package is based on

  - Smear: Expert opinion.*
  - Culture Identification & AST testing: Mode (70% of participants results)
  - Serology: Mode (70% of participants results)

- Statistical Analysis of Data: Scoring of >80% is considered as satisfactory. Scoring of <80% is considered as unsatisfactory for a particular PT scheme. The laboratory must then undertake retraining with appropriate documentation.
- Design of PT scheme: IAMM EQAS New Delhi consists of 3 rounds/year (March, July and November). Each round comprises:
  1. Smear
  2. Culture
  3. Serum specimen for serological testing.

Note: Kindly send your feedbacks for us to do better.

Best wishes till we meet again in next edition, enjoy online experience.

Jai Hind.

----------End of the report----------

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