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Comparison of platform technologies for assaying antibody to Ebola virus

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ABSTRACT

Background: The recent Ebola outbreak in West Africa led to the use of a variety of different platform technologies for assaying antibodies because of the difficulties of handling the live virus. The same types of method could be applied rapidly to other infections when they emerge. There is a need to compare quantitative results of different assays, which means that the assays must measure similar parameters and give comparable results.
Methods: A collaborative study was carried out to establish an International Reference Reagent through WHO. Nine samples were sent to 16 laboratories and the results from 22 different assays compared to those obtained by neutralisation assays using the wild type virus.
Findings: Quantitative correlation with the wild type neutralisation assays was very variable but generally poor, with only five of the twenty-two assays giving a correlation coefficient of 0.7 or greater; the five best assays included methods based on wild type and VSV pseudotype neutralisation and ELISA. They could be applicable to other rapidly emerging diseases. The remaining assays including neutralisation of lentiviral pseudotypes need further development.
Interpretation: The assay platform should be chosen with care to ensure that it is fit for purpose. Many of the assays were not suitable for quantitation of antibody levels, a finding that is not surprising given the urgency with which they had to be implemented but some may be of generic value. Antibody titres in samples from a vaccine trial were comparable to those from convalescent patients or lower.
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1. Background

The need for rapid responses to emerging diseases has assumed greater significance in the wake of the outbreak of Ebola virus disease in West Africa and the current concern over Zika virus. One approach is to develop quantitative platform technologies that can be generally applied for diagnostic and other purposes, including methods for assaying antibody levels to assess the potency of immunological therapeutics such as convalescent plasma and immunoglobulin. Such methods would also be applied to evaluate clinical trials and in clinical diagnosis or serological surveys. The validation and comparison of such assays is outside the normal commercial and regulatory process because by definition they are a response to an emergency where time is of the essence, so there is little information on how the methods compare in a quantitative manner. This paper describes a comparison of a range of assays for antibody to Ebola virus (EBOV) emerging from a project to establish reference reagents under the auspices of WHO.

WHO recorded twenty-four outbreaks of Ebola disease in Africa from 1976 to 2013 with a global total of 1716 cases. The latest Ebola epidemic in Western Africa started in 2014 and up until December 20th 2015 it had resulted in 15,249 cases and 11,315 deaths making it the largest and most significant on record [1]. There were no fully validated commercial assays available because previous outbreaks were sporadic and small scale. Assays for antibody that did not involve working with live Ebola virus had been developed; they included the use of pseudotype neutralisation and ELISA based on expressed recombinant glycoprotein. The relationship between the results of different assays is not clear and given that for example the antibody content of therapeutic materials such as whole plasma or immunoglobulins may be central to their efficacy this is a matter of concern. This paper describes a comparison of assays and analytes used in the collaborative study

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leading to the establishment of the first reference reagent for antibodies to Ebola virus by WHO in October 2015. The full data are to be found in the report to ECBS available on the WHO website [2].

While the objective of the WHO collaborative study was to identify the most suitable sample to serve as the reference, the study also provided the opportunity to compare the performance of the different platforms using the same samples.

2. Materials and methods

2.1. Samples

The nine samples included in the study are listed in Table 1 and were similar to materials that might be used in therapy.

Full details are given in the WHO report[2]. Three were plasma preparations from repatriated convalescent patients, one from Norway (sample 43), one from the USA (sample 79) and one from the UK (NHSBT) (sample 28). Plasma from a normal blood donor in the UK was used as one negative control (sample36). No plasma from West Africa was available at the time of the study. Unlike most patients in West Africa all of the repatriated patients had received sophisticated nursing care and chemotherapeutic or immunological treatments such as Zmapp, a cocktail of human monoclonal antibodies to the Ebola virus glycoprotein, and it is conceivable that the antibodies measured could have been influenced by this; for instance some of the therapeutic antibodies might have persisted despite the time between onset of disease and donation of the plasma. In immunoblots all possessed antibodies to viral proteins in addition to the glycoprotein indicating that antibodies resulting from infection were present. The reference reagent established by WHO is sample 79. The patient who donated sample 79 plasma did not receive monoclonal antibody but did receive other treatment.

Other samples included high and low titre pools of serum obtained from participants in a vaccine trial involving chimp adeno3 vectored EBOV Mayinga glycoprotein followed by vaccinia (MVA) vectored glycoprotein from EBOV Sudan, and Tai forest and Marburg virus. Unfortunately insufficient material was available for these samples to be assayed by all methods. Finally material was obtained from transchromosomal bovines expressing the genes required to produce human immunoglobulin. The animals were immunized with DNA encoding the glycoprotein gene from Zaire 95 and Sudan strains (sample 88) or with a virus like particle formed from the Zaire 2014 Ebola glycoprotein (sample 31), or unimmunised (sample 9). The bovine derived material was highly purified human IgG and immunoglobulin treatment could also be an option for therapy or short term prophylaxis.

Table 1
Samples distributed in the collaborative study.

EBOV Ab sample code	Sample name	Preparation
9	Tc Bovine IgG (negative)	1 mg/mL in sterile buffer#
36	NHSBT EBOV Ab Negative Plasma	SD-extracted
28	NHSBT EBOV Convalescent Ab	SD-extracted
43	Norwegian EBOV Convalescent Ab	SD-extracted
79	American Red Cross EBOV Convalescent Ab	SD-extracted
31	Tc Bovine IgG (immunized with recombinant rGPZaire2014)	1 mg/mL in sterile buffer#
88	Tc Bovine IgG (immunized with Zaire95 + Sudan GP DNA)	1 mg/mL in sterile buffer#
58	Vaccinees Plasma Pool (high)	Plasma pool
64	Vaccinees Plasma Pool (low)	Plasma pool

Abbreviations: NHSBT = National Health Service Blood and Transplant; SD = Solvent-detergent.
PBS-Ca2 + -Mg2+; 5% human serum albumin.

2.2. Assay methods

The range of assays used is given in detail elsewhere [2] and is summarised in outline in Table 2. Four participants performed neutralisation assays using infectious Ebola virus under high containment. Six laboratories performed neutralisation assays using pseudoviruses in which the glycoprotein was expressed on another particle; three involved lentiviral pseudotypes, two vesicular stomatitis virus pseudotypes and one an Ebola virus like particle. The readout of the assays varied. Eight laboratories used different versions of ELISA; one (laboratory 5) used two separate formats and one (laboratory 16) four separate formats. All were directed against the glycoprotein, the likely target of biologically active, neutralising, antibodies. An indirect immunofluorescence assay using wild type virus (IFA) (laboratory 14) and a western blot assay (laboratory 4) were also used in the study. The data from these two laboratories were not strictly quantitative and are not considered further here although the western blot data were used to confirm that the presence of antibodies to specific antigens.

2.3. Expression of results

Most laboratories performed three separate assays and generally each assay included replicates. However insufficient data were available from this study to perform meaningful statistical analysis on intra-laboratory variation. It was also clear that some of the assays were modified between runs to improve sensitivity so that agreement between separate runs was sometimes poor. Given the rapidity with which some of the assays were implemented, full scale validation, as required for a commercial kit, was not to be expected. Results for the neutralisation and pseudo-neutralisation assays were expressed as the median of the 50% end point titres submitted. The results from the ELISA were obtained from dilution series of each samples and were in very different formats. The potencies were therefore determined by parallel line analysis and expressed relative to the results obtained with the highest titre human convalescent plasma sample (sample 79, which was later established by WHO as the first reference reagent). The samples taken from sources unimmunised or unexposed to Ebola did not give dose response curves and were scored as negative except for the human sample 36 when assayed by assayed 16c, d and e. Scatter plots for the results of different assays were generated. The small number of data points meant that the calculated correlation coefficients did not achieve statistical significance. In many cases the points were not evenly distributed over the range of readouts with positive signals being clustered at the high end. The correlation coefficients were therefore calculated with and without the negative sample results to clarify the extent to which the assays were quantitative when testing positive samples. The results including all samples showed the qualitative value of the assays while those including just the known positive samples indicated whether they could be used quantitatively.

3. Results

The results of all assays are summarised in Table 2.

3.1. Neutralisation with live Ebola virus

Of the four laboratories performing neutralisation assays with the wild type virus, two (11a and 12b) submitted comparable results. Laboratory 2 found all samples negative except for sample

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