The efficacy of simulated solar disinfection (SODIS) against coxsackievirus, poliovirus and hepatitis A virus

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ABSTRACT

The antimicrobial activity of simulated solar disinfection (SODIS) against enteric waterborne viruses including coxsackievirus-B5, poliovirus-2 and hepatitis A virus was investigated in this study. Assays were conducted in transparent 12-well polystyrene microtitre plates containing the appropriate viral test suspension. Plates were exposed to simulated sunlight at an optical irradiance of 550 Wm^{-2} (watts per square metre) delivered from a SUNTEST™ CPS+ solar simulator for 6 hours. Aliquots of the viral test suspensions were taken at set time points and the level of inactivation of the viruses was determined by either culture on a HeLa cell monolayer for coxsackievirus-B5 and poliovirus-2 or by utilising a chromogenic antibody-based approach for hepatitis A virus. With coxsackievirus-B5, poliovirus-2 and hepatitis A virus, exposure to SODIS at an optical irradiance of 550 Wm^{-2} for 1–2 hours resulted in complete inactivation of each virus. The findings from this study suggest that under appropriate conditions SODIS may be an effective technique for the inactivation of enteric viruses in drinking water. However, further verification studies need to be performed using natural sunlight in the region where the SODIS technology is to be employed to validate our results.

Key words | drinking water, viral inactivation, virus

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INTRODUCTION

Solar disinfection (SODIS) has been demonstrated to be an efficient, low technology and cost-effective method for reducing the incidence of gastrointestinal infection in communities where access to clean drinking water is limited or absent (Conroy *et al.* 1996, 1999, 2001). The generation of safe drinking water using SODIS is now an established technique in many communities where water is collected into transparent plastic bottles which are then placed in strong direct sunlight for at least 6 hours prior to consumption (McGuigan *et al.* 1998). Besides SODIS there are a variety of methods available for the sterilisation of drinking water including chlorine and iodine tablets (Heiner *et al.* 2011), boiling (Psutka *et al.* 2011) and sodium dichloroisocyanurate (NaDCC) tablets (Morgenthau *et al.* 2012). While these methods can be effective, the associated fuel costs for boiling water can prevent its widespread use and the unpleasant taste of water treated with halogen-based tablets can make compliance an issue. The attraction of SODIS is it makes use of freely available solar energy and it does not affect the taste of the water.

The observed antimicrobial activity is achieved through the synergistic activity of UVA in the 320–400 nm range and solar heating (Wegelin *et al.* 1994). In the laboratory, simulated SODIS has been shown to inactivate pathogenic bacteria, fungi, protozoa and viruses such as poliovirus and the MS2 bacteriophage (Walker *et al.* 2004; Lonnen *et al.* 2005; Heaselgrave *et al.* 2006; Heaselgrave & Kilvington 2010). The exact mode of action by which SODIS exerts its antimicrobial activity is not completely understood but recent studies with bacteria have demonstrated that UVA exposure causes protein oxidation and aggregation leading to the disruption of metabolic function and accelerated cell ageing (Bosshard *et al.* 2010b). Specifically, it has been shown that the enzyme components of the respiratory chain including NADPH and succinate oxidases are

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affected, resulting in the inhibition of adenosine triphosphate (ATP) generation (Bosshard *et al.* 2010a).

In this current study, we have investigated the efficacy of simulated SODIS against a variety of waterborne viral pathogens from the *Picornaviridae* family including poliovirus, coxsackievirus and hepatitis A virus. Poliovirus is the causative agent of poliomyelitis, an acute viral gastrointestinal infection which can cause damage to the central nervous system, resulting in muscle weakness, paralysis and potentially death (Minor 2012). In 1988, the World Health Organization (WHO) initiated the Global Polio Eradication Initiative (www.polioeradication.org) and since then polio cases have decreased rapidly from an estimated 350,000 cases across 125 endemic countries in 1988, to only 1,349 cases in 2010 (WHO 2011). Although now largely eradicated through the introduction of mass immunisation campaigns, four countries, including Afghanistan, India, Nigeria and Pakistan, remain endemic for this disease (WHO 2011). However, while one infectious child remains there is the danger of the re-emergence of this disease. This risk has been highlighted by the re-establishment of transmission in Angola, Chad and DR Congo in 2011 and the occurrence of outbreaks in China and in eight African countries including Ivory Coast, Niger and Mali (CDC 2012). The source of the infections is usually attributed to importation from neighbouring endemic countries (Zarocostas 2011).

Coxsackievirus and other enteroviruses are known to cause a variety of symptoms including hand, foot and mouth disease, aseptic meningitis, polio-like paralysis and encephalitis (Ooi *et al.* 2010). Following the recent decline in cases of poliomyelitis, infections caused by other enterovirus serotypes represent an emerging and increasing threat to human health (Khetsuriani *et al.* 2006; Yang *et al.* 2011). In particular, enterovirus 71 serotype has recently been linked with an epidemic resulting in 490,000 cases in the Asia-Pacific region with 126 deaths (Solomon *et al.* 2010) and coxsackievirus B5 was associated with 1,351 cases in Belarus through the consumption of contaminated drinking water (Amvrosieva *et al.* 2006).

Hepatitis A virus causes an acute infection of the liver leading to symptoms such as jaundice, vomiting and abdominal pain (Cuthbert 2001). In areas endemic for hepatitis A infection, sanitation and hygiene conditions are usually poor and as a result most children become infected in early childhood when symptoms are milder or absent (Franco *et al.* 2012). Problems occur in areas where sanitation and hygiene conditions are variable and as a result exposure to infection is often delayed until later childhood and adolescence when the infection is more severe (Franco *et al.* 2012). This has resulted in a number of large outbreaks among susceptible populations in developing countries (Franco *et al.* 2012). Infection can be easily prevented through the use of an available vaccine but despite this there are approximately 1.4 million cases of the disease annually through the ingestion of faecally contaminated food or water (WHO 2008).

Enteroviruses and hepatitis A virus are spread via faecal–oral transmission and are associated with poor sanitation, poor personal hygiene practices and the lack of availability of clean drinking water. Transmission of these infections can be reduced by improving sanitation and through the treatment of water prior to drinking and use in food preparation. In this present study, we have investigated the inactivation of waterborne enteric viruses when exposed to simulated SODIS and the demonstration that SODIS is effective against these pathogens has prompted this report.

MATERIALS AND METHODS

Chemicals and media

Chemical reagents were obtained from the Sigma Chemical Company, Dorset, UK. Culture media was obtained from Oxoid (Basingstoke, UK) and Becton Dickinson and Company (Oxford, UK).

Pathogens and cell lines

Poliovirus-2 (PV-2) strain (NCPV 503) was obtained from the National Collection of Pathogenic Viruses (Health Protection Agency, Porton Down, UK). Coxsackievirus-B5 (CV-B5) strain (VR-105, Faulkner) was obtained from the American Type Culture Collection (LGC Standards, Teddington, UK). The enteroviruses were maintained in the human HeLa (Hep2 derivative) cell line (ECACC

85011412) obtained from the European Collection of Cell Cultures (Health Protection Agency, Porton Down, UK). Hepatitis A virus (HAV-1) strain (VR-1402, HM175/18f) was obtained from the American Type Culture Collection (LGC Standards, Teddington, UK). Hepatitis A virus was maintained in the FRhK4 monkey kidney cell line (CRL-1688) obtained from the American Type Culture Collection (LGC Standards, Teddington, UK). Both mammalian cell lines were incubated at 37 °C (5% CO_2) in Dulbecco's minimum essential medium (DMEM) supplemented with 10% (v/v) heat-inactivated foetal calf serum. For the propagation of the viruses, the same DMEM was used but with the omission of the foetal calf serum. Viruses were titred by determining the tissue culture infective dose (TCID50/ml) using the Spearman-Karber method (Hamilton *et al.* 1977) as described previously (Alotaibi & Heaselgrave 2011).

SODIS assay method

All assays were conducted in transparent 12-well tissue culture polystyrene (PS) microtitre plates with matching lids (Helena Biosciences, Gateshead, UK). Test organisms were suspended and diluted in laboratory grade ultra-pure H_2O immediately before use to give a concentration of 1×10^7/ml viral particles. Ultra-pure water was selected as the aim of this study was to demonstrate if SODIS was theoretically capable of inactivating viruses under ideal laboratory conditions. Using a pipette, 3 ml of the viral suspensions were added to the wells of the microtitre plates. The plates, with the lids in place, were exposed to simulated sunlight in a SUNTEST™ CPS+ solar simulator (Atlas MTS, Bicester, UK). The simulator contains a 1.5 kW xenon bulb enclosed within the test apparatus and has a thermostatically controlled test chamber to maintain a constant chamber temperature throughout the experiment. Samples were exposed at an optical output irradiance of 550 Wm^{-2} (watts per square metre) calibrated between 290 and 800 nm in the UV and visible wavelengths. This intensity corresponds to the value of 870 Wm^{-2} global irradiance calibrated in the range 200–4,000 nm used in our previous publication using a different lamp system (Heaselgrave *et al.* 2006). The SUNTEST™ CPS+ solar simulator contains a dual filter system which consists of a coated quartz glass filter which reflects a portion of the intense infrared radiation produced by the xenon lamp above 800 nm and a UV special glass filter which gives a better spectral match to outdoor natural sunlight. The SUNTEST™ CPS+ lamp and dual filter system used in this study meets the specification of the European Cosmetics Association (COLIPA 2009) standard which is used for the determination of UVA protection provided by sunscreen (Matts *et al.* 2010). In addition, the filter system meets the specification outlined in the Solar Spectral Irradiance technical report (CIE-085, 1989) published by the International Commission on Illumination (CIE). Control plates were wrapped in aluminium foil to prevent light exposure and placed in the test chamber next to the test samples. The temperature of the test solution inside the solar simulator reached up to 45 °C and so for all experiments an additional test sample containing each virus was incubated at 45 °C in the dark and then tested alongside the other samples.

Inactivation assays: culture-based method for enteroviruses

The inactivation of poliovirus-2 and coxsackievirus-B5 was determined using a culture-based method looking for virally induced cytopathic effect (CPE) on a HeLa monolayer as described previously (Heaselgrave *et al.* 2006). At timed intervals of 0, 1, 2, 4 and 6 hours, the test and control wells were mixed by pipetting. Then quadruplet 20 µl volumes were removed and added to the wells of a 96-well microtitre plate (Helena Biosciences, Gateshead, UK) containing a confluent monolayer of the appropriate cell line for each virus suspended in Dulbecco's modified Eagle's medium (DMEM). Tenfold serial dilutions were made across the plate which was then incubated at 37 °C (5% CO_2) to allow for viral replication. For the enteroviruses the plates were examined daily for 7 days using an inverted microscope (×200) to look for the presence of virally induced CPE on the HeLa cell monolayer.

Inactivation assays: antibody-based method for hepatitis A virus

Hepatitis A virus causes only minimal CPE on the FRhK4 cell line and so a chromogenic antibody-based approach was utilised to determine viral inactivation. The method

was modified from a previously published method to include a peroxidase conjugated antibody (Counihan *et al.* 2006). Briefly, the 96-well plates were processed as for the other viruses but the plates were kept in the incubator for 7 days before processing. After 7 days, the plates were removed from the incubator and the DMEM was aspirated from each well. The FRhK4 cells were fixed and permeabilised with a mixture of formalin containing 0.5% (v/v) Triton-X and allowed to stand for 20 min. The mixture was aspirated from the cells and the monolayer was washed twice using Dulbecco's phosphate buffered saline (DPBS) containing 0.5% (v/v) Triton-X. The wells were then blocked to reduce non-specific binding using a solution of DPBS containing 5% (w/v) skimmed milk and left for 1 hour. Following this, the wells were aspirated off and filled with a suspension containing the primary antibody diluted 1:200 in DPBS containing 3% (w/v) skimmed milk and 0.3% (v/v) Tween® 20. The primary antibody was a mouse anti-hepatitis A IgG_{2A} monoclonal #01885501 (CSL Ltd, Melbourne, Australia) antibody. The plates were incubated overnight at 4 °C on a shaking platform. The next day the wells were aspirated and washed (×4) with wash buffer (DPBS with 0.3% (v/v) Tween® 20) for 5 min. The wells were then filled with a suspension containing the secondary antibody diluted 1:1,000 in the buffer as indicated for the primary antibody and incubated for 1 hour at 25 °C on a shaking platform. The secondary antibody was an anti-mouse IgG peroxidase conjugate #A3673 (Sigma, Dorset, UK). Next the wells were aspirated and washed (×4) with wash buffer (DPBS with 0.3% (v/v) Tween® 20) for 5 min. Any remaining liquid was aspirated off and the wells were filled with KPL TrueBlue substrate (Insight Biotech, Wembley, UK). Virally infected cells were then identified by the development of a blue precipitate within the monolayer which could be viewed using an inverted microscope (×200).

Data and statistical analysis

The level of viral inactivation for each time point was calculated using a most probable number approach using Spearman-Karber computations (Hamilton *et al.* 1977). The level of viral inactivation was plotted with standard error of the mean for each time point according to the formula $\log (N_t/N_0)$ where N_t is the viable count at an experimental time point and N_0 is the initial viable count at the start of the experiment. Statistical analysis was performed using one-way analysis of variance (ANOVA) of data from triplicate experiments on the InStat statistical software package (GraphPad, La Jolla, USA).

RESULTS

The efficacy of simulated sunlight at an optical output irradiance of 550 Wm^{-2} against coxsackievirus (CV-B5), poliovirus (PV-2) and hepatitis A virus is shown in Figures 1–3. In all three figures, the $\log_{10}$ levels of viral inactivation are mean values from triplicate experiments displayed with standard errors of the mean (SEM).

In Figures 1 and 2, with coxsackievirus and poliovirus, respectively, a >4 $\log_{10}$ inactivation of viruses was observed by the 1 hour time point under simulated SODIS conditions. This indicated complete inactivation of both viruses as no CPE was observed on the HeLa cell monolayer. With the dark control, in which the samples were placed inside the solar simulator but were protected from the light, no viral inactivation was observed over the 6 hours of the experiment. With the temperature control, where the viral sample was incubated at 45 °C for the duration of the experiment in the dark, a 1.7 $\log_{10}$ and a 1.3 $\log_{10}$ of viral inactivation was observed, respectively, after 6 hours.

In Figure 3, with hepatitis A virus, a 4 $\log_{10}$ inactivation of viruses was observed by the 2 hour time point under simulated SODIS conditions. This indicated complete inactivation of virus as no further blue precipitate was observed on the mammalian cell monolayer with the TrueBlue substrate. Over the 6 hours of the experiment, no viral inactivation was observed with the dark control or temperature control samples.

DISCUSSION

Throughout many parts of the world, the lack of access to suitable drinking water is a major health concern as the consumption of drinking water contaminated with waterborne human pathogens including bacteria, protozoa and viruses is a major cause of morbidity and

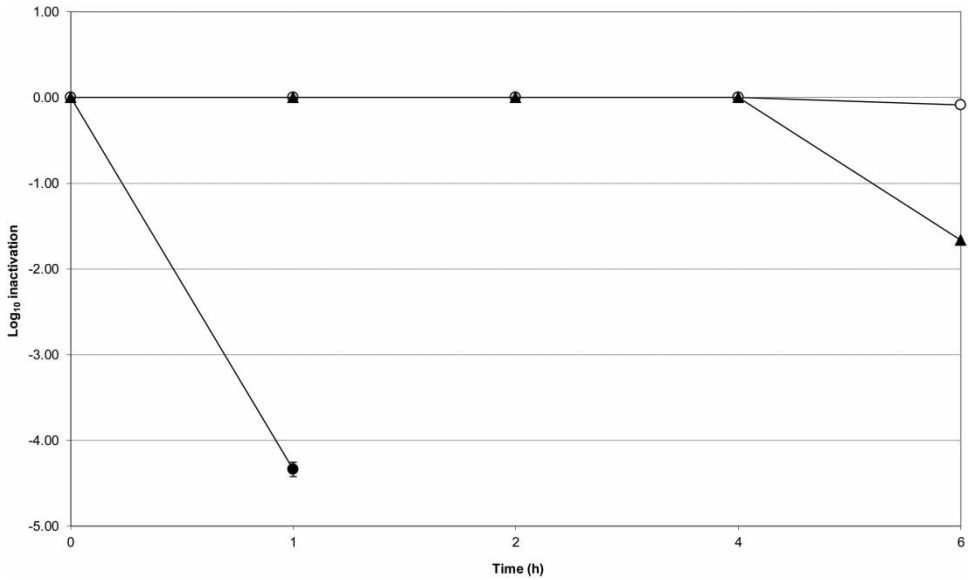

Figure 1 | Efficacy of simulated SODIS against coxsackievirus-B5 using an optical irradiance of 550 W/m^2 on the Atlas CPS+ solar simulator; (•) SODIS, (○) dark control, (▲) 45 °C temperature control. Error bars show standard error of the mean from triplicate experiments.

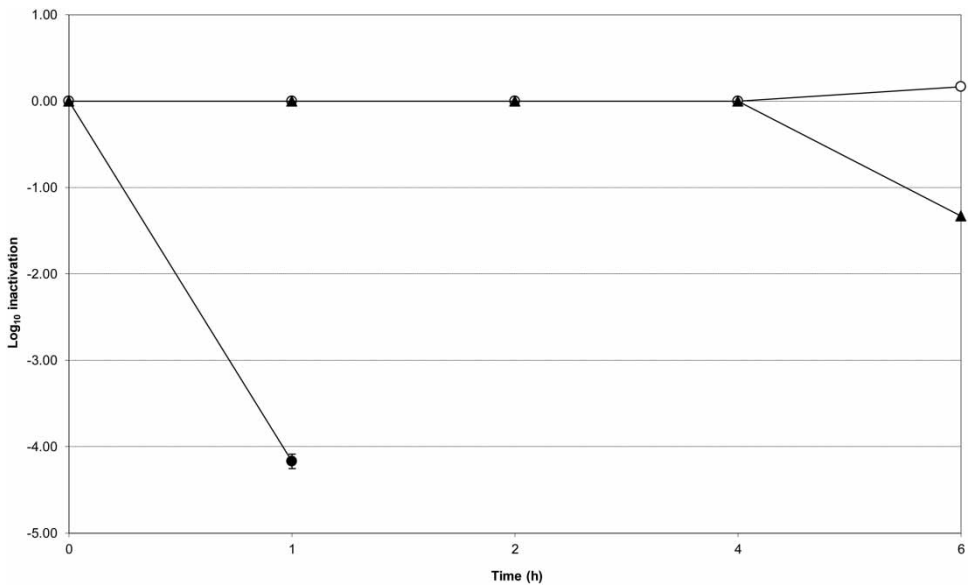

Figure 2 | Efficacy of simulated SODIS against poliovirus-2 using an optical irradiance of 550 W/m^2 on the Atlas CPS+ solar simulator; (•) SODIS, (○) dark control, (▲) 45 °C temperature control. Error bars show standard error of the mean from triplicate experiments.

mortality (Park *et al.* 2010; Baldursson & Karanis 2011; CDC 2011). Two recent studies from South Africa and Cambodia have demonstrated SODIS to be a highly effective tool for the reduction in diarrhoeal disease in compliant communities (Du Preez *et al.* 2010; McGuigan *et al.* 2011). Therefore the implementation of SODIS initiatives in communities where there is the need for improved water sterility and which have suitable weather conditions may be enormously beneficial. In this study we have investigated the efficacy of simulated SODIS against

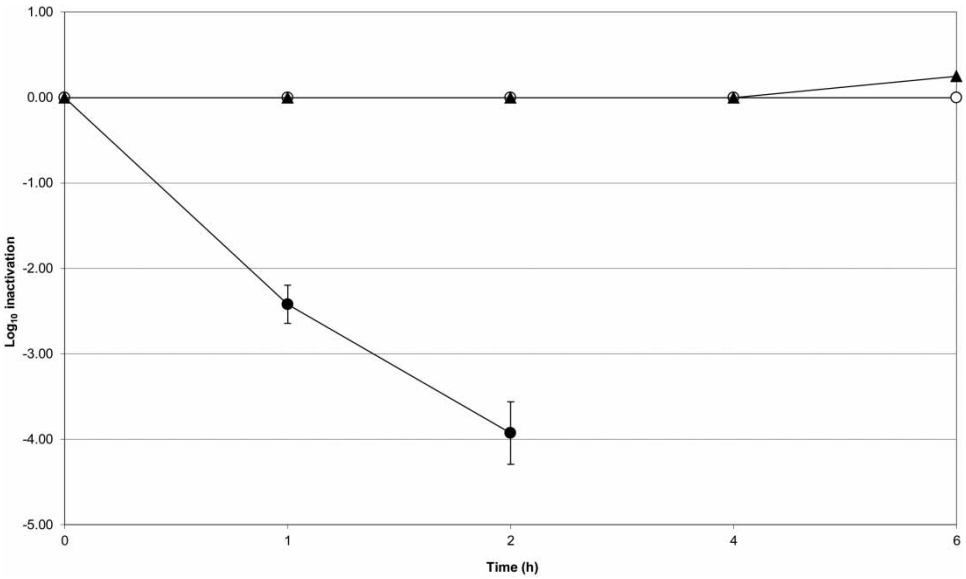

Figure 3 | Efficacy of simulated SODIS against hepatitis A virus using an optical irradiance of 550 W/m^2 on the Atlas CPS+ solar simulator; (•) SODIS, (○) dark control, (▲) 45 °C temperature control. Error bars show standard error of the mean from triplicate experiments.

three waterborne viral pathogens to determine if SODIS has the potential to be effective against these enteric viruses.

We have previously demonstrated the efficacy of SODIS against a variety of waterborne protozoan pathogens including *Entamoeba*, *Giardia*, *Naegleria*, *Acanthamoeba* and the enteric virus poliovirus (Heaselgrave *et al.* 2006; Heaselgrave & Kilvington 2011). However, this is the first study to report the ability of simulated SODIS to achieve the rapid and complete inactivation of poliovirus, hepatitis A virus and coxsackievirus within 1–2 hours. Previous studies performed with coxsackievirus using a low-powered SODIS lamp were only able to achieve the partial inactivation of the virus (Alotaibi & Heaselgrave 2011). The complete inactivation of poliovirus seen in this study by the 1 hour time point is significantly faster than that reported previously (Heaselgrave *et al.* 2006). In the previous study, we exposed the poliovirus to simulated SODIS at a comparable intensity but on that occasion the time required to achieve complete inactivation was slower at 2 and 4 hours at temperatures of 25 and 40 °C, respectively. The explanation for the faster rate of viral inactivation seen in this present study is the higher water temperature of 45 °C, as solar heating has been shown to work synergistically with solar radiation to enhance the SODIS process (Rijal & Fujioka 2001). The reason for selecting the higher temperature was based on our experiences when performing SODIS under natural sunlight in Spain when we regularly observed water temperature in excess of 45 °C (unpublished observation) and so we felt that this was closer to conditions under natural sunlight.

The observation that SODIS is a rapid technique for the inactivation of viruses is contradicted by a recent study which used the MS2 phage as a model organism (Fisher *et al.* 2012). The authors in that study reported that the time required for the 3 $\log_{10}$ inactivation of the virus in a PS and polyethylene terephthalate (PET) container was approximately 20 and 33 hours, respectively (Fisher *et al.* 2012). The same group has previously determined that UVB light was required for the inactivation of the MS2 phage and poliovirus (Love *et al.* 2010; Fisher *et al.* 2011) and stated that the poor level of UVB transmission in PS and PET is responsible for the extended disinfection time of the MS2 phage (Fisher *et al.* 2012). However, all of the studies in this current study were performed in PS containers and we were able to achieve rapid inactivation of the viruses in 1–2 hours. Furthermore, a previous study achieved a 3.5 log inactivation of the MS2 phage contained within a PET container after 6 hours exposure to SODIS (Walker *et al.* 2004). The exact reason for the difference in

results between the studies is unclear but it seems possible that the explanation lies in differences in temperature between studies. The experiments in this current study were performed in a laboratory environment and were maintained at a constant temperature of 45 °C, whereas the Fisher *et al*. (2012) study performed experiments under natural conditions and reported significant variation in temperature over the course of the experiments.

CONCLUSIONS

In this present study, we have been able to show that the simulated SODIS is effective against waterborne pathogens including poliovirus, hepatitis A virus and coxsackievirus and can achieve complete inactivation within 1–2 hours. The findings from this study suggest that under appropriate conditions SODIS may be an effective technique for the inactivation of enteric viruses in drinking water. However, further verification studies need to be performed using natural sunlight in the region where the SODIS technology is to be employed in order to validate our results.

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